BRANCHED CHAIN FATTY ACIDS (BCFA) IN NATURE: FISH, FERMENTED FOODS, AND SEA LION VERNIX CASEOSA

A Dissertation

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BRANCHED CHAIN FATTY ACIDS (BCFA), POLYUNSATURATED FATTY ACIDS IN FRESHWATER FISH, FERMENTED ASIAN FOODS AND BCFA RICH VERNIX IN SEA LIONS

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Branched chain fatty acids (BCFA) are major components of the western food supply, constituting 500 mg per day mean intake in Americans mainly from dairy, beef, and other ruminant products, and a major component of the first solid meal of human fetuses. We sought to establish the degree to which fish and fermented foods may contain BCFA, and to investigate anecdotal reports of BCFA-rich vernix caseosa in sea lions. Twenty-seven wild fishes collected from fresh waters in the northeastern United States were analyzed. BCFA was only 1% ± 0.5% (mean ± SD) of total fatty acids, contributing only a small amount of BCFA per serving to the diet. Surprisingly, one serving of these fishes contributes much higher amounts of EPA + DHA than generally appreciated (107 mg to 558 mg). This study also revealed that odd chain fatty acids are associated with fish and that the ratio of high 15:0 to 17:0 is indicative of a fish origin whereas the reverse is known for dairy, suggesting a possible biomarker. Though dairy is much less commonly consumed in Asian than in Western countries, a recent study reported similar BCFA in breast milk collected from Asian and American mothers. Numerous well known fermented

Asian foods were acquired and tested to determine if they accumulate BCFA during fermentation. Fermented soy known as nātto and fermented shrimp paste had high BCFA as a percent of fatty acids, 1.71±0.17% and 3.18 ± 0.14% BCFA, respectively. The major BCFA in nātto are iso-14:0, iso-15:0, anteiso-15:0, iso-16:0, iso-17:0 and anteiso-17:0, substantially recapitulating the BCFA profile of fluid milk. Consuming one typical serving (90 g) of nātto provides 117 mg of BCFA while the shrimp paste, used more as a flavoring agent, had 8.8 mg BCFA per serving (16g). Nātto and shrimp paste are the first fermented foods identified with BCFA approaching or exceeding that of milkfat (2.0% BCFA). Vernix caseosa, rich in BCFA, is the richest BCFA source known to be a nutritional source for humans, but is unknown in any other species. Analysis of GI tract contents of newborn California sea lions (CSL) show high levels of BCFA and squalene. CSL are thus the second species apart from humans now known to biosynthesize vernix in late gestation, delivering BCFA and squalene to the fetal GI tract. These data are further evidence for the importance of BCFA as likely modifiers of microbiota and GI metabolism.

BIOGRAPHICAL SKETCH

Donghao Wang was born in Shenzhen, China to Yongjun Wang and Zhaohua Li, both came to the newly established city after graduation from their college. After graduated from Shenzhen High School, Donghao went to Guangzhou Jinan University where he studied Food Quality and Safety for four years. He finished as the top student in his class and came to Cornell University for his MPS degree in Food Science Department. He was doing research in Professor J. Thomas Brenna’s lab during his study. After a year, he began his Ph.D in Dr. Brenna’s lab where he stayed for another four years and met his wife, Zhen Wang there. They got married in 2016 in the witness of their parents, friends and advisor.

ACKNOWLEDGMENTS

My Ph.D study cannot go smoothly without the generous assistance of many people. First I need to thank my advisor Dr. Brenna for all his support and Rinat Ran-Ressler for her training and guidance. All my other lab mates are always been a priceless source and trustful. My parents are always there for me when I am excited or depressed. I can’t express how much I love them. My wife, Zhen Wang cares for me all the time since we met and also provide academic support to me when I feel lost. There are also friends and classmates who I really need to thank. They made my stay on campus so joyful and meaningful. I am pretty sure that I am going to miss Cornell and all the people I met here. Thank you all!

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LIST OF ABBREVIATION

ARA, arachidonic acid

BCFA, branched chain fatty acids

CSL, California sea lion

CV, coefficient of variance

DHA, docosahexaenoic acid

EI, electron ionization

EPA, eicosapentaenoic acid

FAEE, fatty acid ethyl esters

FAME, fatty acid methyl esters

FID, flame ionization detector

GC, gas chromatograph

GI, gastrointestinal

LCPUFA, long chain polyunsaturated fatty acids

MS, mass spectrometry

MUFA, monounsaturated fatty acids

NEC, necrotizing enterocolitis

SD, standard deviation

PUFA, polyunsaturated fatty acids

SFA, saturated fatty acids

CHAPTER 1

Saturated branched chain, normal odd-carbon-numbered, and n-3 (omega-3) polyunsaturated fatty acids in freshwater fish in the northeastern United States*

Abstract

The fatty acid profiles of wild freshwater fish are poorly characterized as a human food source for several classes of fatty acids, particularly for branched chain fatty acids (BCFA), a major bioactive dietary component known to enter the U.S. food supply primarily via dairy and beef fat. We evaluated the fatty acid content of 27 freshwater fish species captured in the northeastern U.S. with emphasis on the BCFA and bioactive polyunsaturated fatty acids (PUFA) most associated with fish, specifically n-3 (omega 3) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Mean BCFA content across all species were 1.0±0.5% (mean ± SD) of total fatty acids in edible muscle, with rainbow smelt (*O. mordax*) and pumpkinseed (*L. gibbosus*) the highest at >2% BCFA. In comparison, EPA+DHA constituted 28%±7% of total fatty acids. Across all fish species, the major BCFA were *iso*-15:0, *anteiso*-15:0, *iso*-16:0, *iso*-17:0 and *anteiso*-17:0. Fish skin had significantly higher BCFA content than muscle tissues, at 1.8%±0.7% but lower EPA and DHA. Total BCFA in fish skins was positively related with that in muscle (r^2=0.6). The straight chain saturates n-15:0 and n-17:0 which have been identified previously as markers for dairy consumption were relatively high with means of 0.4% and 0.6%,

respectively, and may be an underappreciated marker for seafood intake. Consuming a standardized portion, 70 grams (2.5 oz.), of wild freshwater fish contributes only small amounts of BCFA, 2.5-24.2 mg, to the American diet, while it adds surprisingly high amounts of EPA+DHA (107 mg to 558 mg).

* Wang, D. H.; Jackson, J. R.; Twining, C.; Rudstam, L. G.; Zollweg-Horan, E.; Kraft, C.; Lawrence, P.; Kothapalli, K.; Wang, Z.; Brenna, J. T., Saturated Branched Chain, Normal Odd-Carbon-Numbered, and n-3 (Omega-3) Polyunsaturated Fatty Acids in Freshwater Fish in the Northeastern United States. *J Agric Food Chem* **2016**.

Introduction

Finfish are known as a lean source of protein and n-3 (omega 3) long chain polyunsaturated fatty acids (LCPUFA) accessible to both developed and developing countries. Global per capita consumption of fish has increased from 9.9kg to 19.2kg in the past 50 years, and has grown faster than the rate of world population expansion in the most recent decade, owing to the rapid expansion of aquaculture especially in Asian countries [1]. The northeastern United States has many lakes and streams with a variety of native fish species. Common fishes utilized for food include walleye (*S. vitreus*), white perch (*M. Americana*), yellow perch (*P. flavescens*), lake trout (*S. namaycush*), salmon species, and channel catfish (*I. punctatus*) in addition to dozens of other species less commonly consumed, despite potentially being nutritious and palatable.

From a health perspective, omega-3 docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are a main motivation for higher fish consumption. Apparent DHA/EPA deficiencies are corrected by fish consumption and are connected to lower risk of coronary heart disease in a series of prospective observational studies [2]. Omega-3 LCPUFA are also linked to improved cognitive abilities in children [3] and visual acuity in infants [4]. The Dietary Guidelines for Americans recommends 8-12 ounces/week of seafood [5], particularly for pregnant and lactating women because placental and breast milk transfer depletes DHA starting at the beginning of pregnancy [6]. The American Heart Association also recommends seafood consumption, especially for secondary cardiovascular disease prevention.

While the content and health benefits of DHA in fish are widely studied, branched chain fatty acids (BCFA) in fish are less well characterized. BCFA are mostly saturated fatty acids with a terminal propan-2-yl (isopropyl) group (*iso*) or butan-2-yl (*sec*-butyl) group (*anteiso*)[7] (**Figure S1.1**). Those with 14 to 18 carbons in the chain are most common in the U.S. food supply as components of dairy and beef fat [8]. Reports of BCFA levels in fish are typically below 5 % though they vary greatly, from low ranges of 0.3%-1.5% in fish caught near Senegal [9] to a surprisingly high 40% in flathead grey mullet (*M. cephalus*) captured in a mangrove estuary [10]. Chinese carp species cultured for food fish had BCFA ranging from 1.8 to 4% [11], while common carp (*C. carpio*) captured in Madagascar had 4-5% BCFA [12]. Ongoing research shows BCFA are active compounds with bioactivity benefits, such as development of gut microbiota[13], and antitumor [14] effects.

Fish skin is considered an integral part of a food fish in many parts of the world but is less appreciated in the US. As a result, fish skin poses a waste problem in the fish processing industry in the US. However, fish skin can be nutritionally valuable in terms of healthy lipids. Fish skin has more total lipid per unit mass and a significant amount of n-3 LCPUFA [9, 15]. We are aware of only one report of the BCFA content of fish skin, showing about 0.3% *iso*-15:0 and 0.3% *iso*-17:0 in skins of three edible fish from Senegalese coast [9].

Our purpose was to characterize the fatty acid profile of common fish from the New York State area as a representative of the fresh waters of the northeastern US. For the first time, we emphasize the full range of food fatty acids in wild caught freshwater fish, specifically BCFA and odd-carbon-numbered as well as the much better studied omega-3 EPA and DHA. From these data, we quantify BCFA intake from fish and compare it to other diet components and shed new light on the origin of odd chain fatty acids as a biomarker of intake in humans in the context of highly cited associations with cardiovascular disease. We also compare these U.S. fish of known origin to previous reports of very high BCFA levels from fish caught in Asia.

Methods

Sampling

Twenty-seven species of wild fishes were caught in Oneida Lake, Cayuga Lake, Whitney Point Reservoir, the Adirondack region and some creeks in the states of New York and Pennsylvania. Locations and dates of capture, length, sample size and dietary information of each of the fish species are presented in **table S1.1**. Fish are listed from highest to lowest muscle total BCFA content, which will be presented in **table 1.1** below. Fish were identified by fish biologists from Cornell University and the New York State Department of Environmental Conservation. Fish were put into a cooler packed with ice upon capture and transported to Cornell University immediately. All fish were kept at -80°C until processing.

Fatty Acid Analysis

Two hundred mg of fish muscle at the dorsal fin, caudal fin, and belly, and fifty mg of skin, were homogenized and placed into separate glass tubes and extracted and methylated by a modified one-step hydrolysis and methylation procedure, as described previously [16]. Tricosanoic acid (23:0) was added quantitatively and served as internal standard to calibrate areas to mg FA in sample after response correction (Sigma Chemical Company). Reported total fat content reflects fatty acids and without a correction for non-fatty acid lipid components. Fatty acid methyl esters (FAME) were analyzed as previously discussed [17]. Briefly, a BPX-70 capillary column (25m×0.22mm×0.25µm; SGE) with H_2 as carrier gas was installed in a HP 5890 gas chromatograph with a flame ionization detector (GC-FID), which was used for quantitative analysis. A FAME mixture of equal weight (GLC462; Nu-Chek Prep, Inc.) was used to calculate response factors and six BCFA were used as authentic reference standards (*iso*-14:0, *anteiso*-15:0, *iso*- 16:0, *anteiso*-17:0, *iso*-18:0 and *iso*-20:0; Larodan Fine Chemicals AB). **Table S1.2** presents the retention time of a sample and both standards from the same run. Concentrations of all FA are expressed as %, w/w. FAME identities were determined by chemical ionization, electron ionization (EI) mass spectrometry (MS), and BCFA structures were verified by EIMS/MS as described previously [18] using a Varian Star 3400 GC coupled to a Varian Saturn 2000 ion trap MS. Briefly, in MS-2 of the rearranged molecular ion, collisional activation of iso-BCFA yield a characteristic [M-43] ion corresponding to isopropyl cleavage and anteiso-BCFA yield two ions, [M-29] and [M-57], corresponding to cleavage on either side of the methyl branch. The mass spectrometry parameters are as follows. M + ions for BCFAME were isolated for fragmentation in EIMS2 mode. The ionization mode was set to “EI auto mode” using the default parameters set by the Varian Saturn software V5.5.2. Ion preparation parameters were as follows: isolation window 3.0 amu; waveform type residence; excitation storage level was calculated using a q value of 0.215; excitation amplitude was set to 0.80 V. Segment set point parameters were: scan rate 1 s; count threshold 1; emission current 0.5 µA. All spectra were collected under the identical instrument settings, including collision energy (excitation amplitude) and mass isolation window. In our hands, these conditions provided suitable fragments intensities across all BCFAME without the need to customize parameters.

Statistical Analysis

Fatty acid compositions were analyzed with ANOVA and paired sample t tests carried out in JMP Pro 12 software for windows. Specifically, select fatty acids common to all fish species 14:0, 15:0, 16:0, 16:1n-7, *anteiso*-17:0, 17:0, 18:0, 18:1n-9, 18:2n-6, 18:3n-3, 18:4n-3, 20:4n-6, 20:5n-3, 22:6n-3 and total BCFA were investigated with ANOVA to detect differences in tissue types. Three specific fatty acid classes, saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA), were also analyzed for the same purpose. Paired sample t tests between muscle at the dorsal fin and skin were used to verify the fatty acids that showed a significant difference between muscle and skin in the ANOVA test. Coefficient of determination (r^2) and the fitted linear model were obtained in Microsoft Excel. ANOVA was performed to determine the significance of the regression. We also used ANOVA to examine the effects of sampling location, habitat type (Lake or Stream), and foraging guild on percent total BCFA, percent EPA, and percent DHA. We selected models using AIC [19] and found that including multiple variables in models and interactions between variables provided little improvement to model suitability. ANOVA of percent BCFA, percent EPA, and percent DHA by sampling location, habitat type, and foraging guild [20] were performed in R (version 3.2.2). Significance level was set at $p \leq 0.05$ if not otherwise specified.

Results and Discussion

Fatty Acids of Muscle Tissues in 27 Fish Species

Muscle tissues at the dorsal fin of 27 fish species were analyzed for fatty acids profile. **Figure 1.1** is a summary of the main classes of FA present in the analyzed fish. SFA comprised 31±5% (mean±SD) of total FA, with two thirds being palmitic acid (16:0). MUFA were 17±3% and PUFA were highest at 52±6%. Mean BCFA content was 1.0±0.5% of total FA, or 3.2% of SFA. **Figure 1.2** shows that arachidonic acid (ARA, 20:4n-6), EPA (20:5n-3) and DHA (22:6n-3) were the major PUFA. Total EPA+DHA had a mean of 28±7% and ARA was 10±5%. Linoleic acid (18:2n-6) and linolenic acid (18:3n-3) comprised 2.7% and 1.4% of total FA separately. Eicosatetraenoic acid (20:4n-3) and adrenic acid (22:4n-6) were both lower than 1%. The isomers of docosapentaenoic acid (22:5n-6 and 22:5n-3) were 1.9% and 4.0%. Full fatty acid profiles and typical chromatograms from GC-FID and EIMS/MS are presented in the supplement (**Table S1.3 & Figure S1.2**).

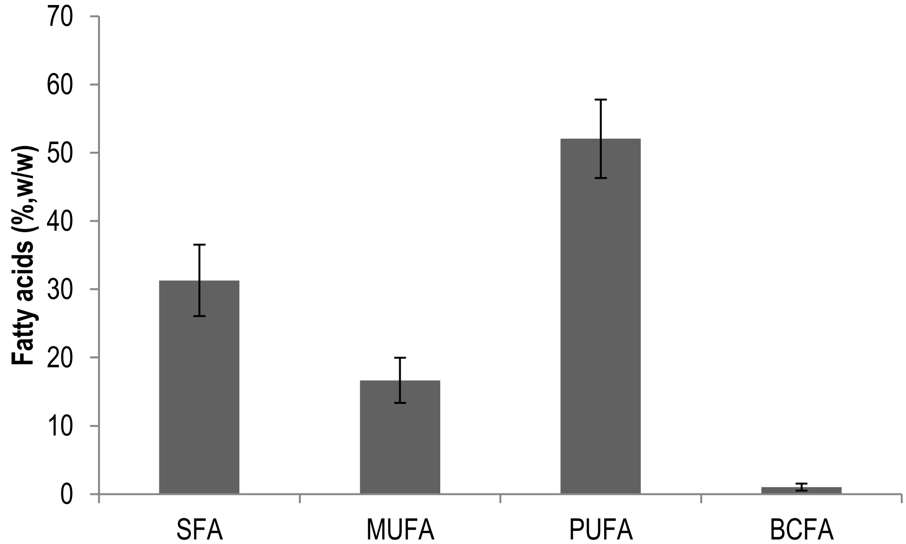


Figure 1. 1 Overall fatty acid (FA) composition (%, w/w; mean ± SD) of 27 fish species in the northeastern United States.

FA were grouped as follow: Saturated fatty acids (SFA); monounsaturated fatty acids (MUFA); polyunsaturated fatty acids (PUFA); branched chain fatty acids (BCFA)

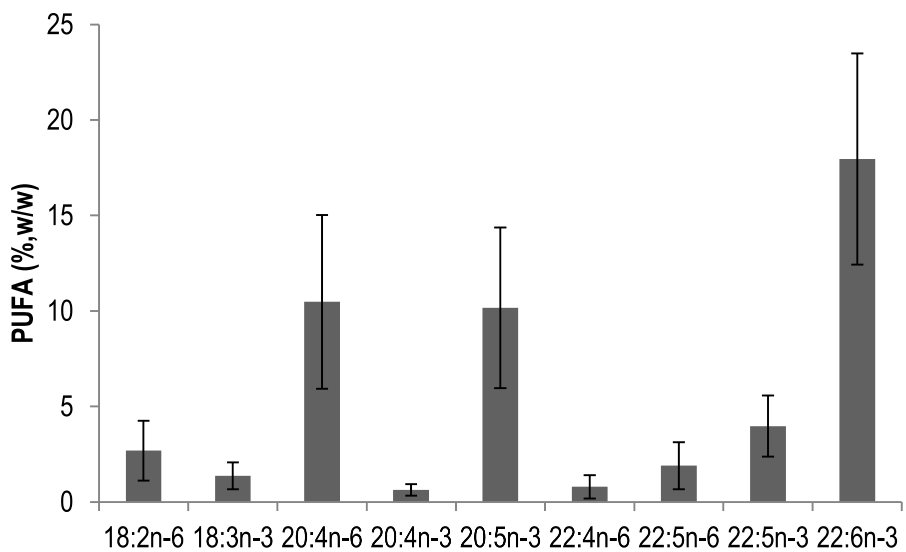


Figure 1. 2 Major polyunsaturated fatty acids (PUFA) composition (>0.5%, w/w of total FA; mean ± SD) of 27 fish species in the northeastern United States

Table 1.1 shows the BCFA chain length distribution and concentrations as well as concentrations of ARA, EPA and DHA in the 27 fish species. Fish BCFA had 13-20 carbons with *iso*-15:0, *anteiso*-15:0, *iso*-16:0, *iso*-17:0, *anteiso*-17:0 comprising about 90% of total BCFA by weight. *Iso*-13 and *anteiso*-13, *iso*-18:0, *anteiso*-19:0 and *iso*-20:0 were also found at low concentrations in some fish. In addition, *iso*-12:0 was detected at trace levels in a few samples (data not shown). Total BCFA mean values ranged from 0.5% to 2.2% of total fatty acids, with about half the species near 1%. Ten fish species that had more than 1% BCFA and rainbow smelt and pumpkinseed exceeded 2% BCFA. All BCFA followed the fragmentation pattern described in the method section.

Table 1. 1 Weight percent for BCFA and major PUFA of 27 fish species caught in the northeastern United States (muscle)

Fatty acid %wt	*iso*-13:0	*ai*-13:0	*iso*-14:0	*iso*-15:0	*ai*-15:0	*iso*-16:0	*iso*-17:0	*ai*-17:0	*iso*-18:0	*ai*-19:0	*iso*-20:0	Total BCFA	ARA	EPA	DHA
Rainbow smelt			0.03	0.46	0.20	0.18	0.29	0.56	0.38	0.06		2.17	5.72	9.97	26.44
Pumpkinseed	0.01		0.01	0.15	0.10	0.16	0.73	0.68	0.03	0.19		2.07	9.91	8.00	15.41
White sucker			0.01	0.15	0.08	0.16	0.61	0.44	0.11	0.04	0.03	1.64	11.64	8.59	14.69
Lake trout				0.13	0.05	0.11	0.65	0.45	0.12	0.06		1.58	5.44	7.25	24.61
Freshwater drum			0.04	0.27	0.05	0.13	0.39	0.18	0.06		0.06	1.19	16.15	14.11	4.95
Alewife			0.01	0.17	0.08	0.13	0.44	0.23	0.06	0.04		1.17	5.43	9.79	15.15
Common shiner	0.03	0.02	0.02	0.16	0.23	0.13	0.27	0.21	0.06			1.13	12.59	11.16	10.16
White crappie			0.07	0.23	0.04	0.10	0.47	0.14			0.07	1.12	15.15	5.77	14.65
Walleye		0.03		0.16	0.04	0.08	0.38	0.20		0.18	0.01	1.08	9.47	6.44	21.46
Channel catfish				0.14	0.04	0.13	0.34	0.24		0.07	0.05	1.01	8.46	8.18	15.66
Greater redhorse			0.01	0.11	0.09	0.09	0.35	0.25		0.09		0.99	5.84	15.43	14.41
Black crappie			0.01	0.12	0.05	0.07	0.43	0.10	0.09	0.08		0.95	17.17	9.14	11.49
Smallmouth bass			0.03	0.16	0.04	0.10	0.38	0.20		0.04		0.95	14.14	4.95	19.38
Golden shiner			0.03	0.10	0.03	0.07	0.55	0.14				0.91	10.49	12.40	16.74
Slimy sculpin	0.02	0.01	0.04	0.18	0.15	0.03	0.27	0.20	0.02			0.91	8.01	17.36	17.78
Brown bullhead			0.04	0.10	0.11	0.06	0.39	0.12			0.05	0.88	14.02	7.77	16.52
Redbreast sunfish				0.21	0.04	0.12	0.26	0.23				0.87	10.60	6.20	18.69
Blacknose dace	0.01	0.01	0.01	0.10	0.11	0.09	0.16	0.22	0.02			0.74	4.50	11.62	24.04
Rock bass				0.10	0.03	0.07	0.27	0.11	0.09	0.04	0.01	0.72	7.88	9.69	24.94
Longnose dace	0.05	0.09	0.02	0.08	0.06	0.07	0.10	0.18	0.07			0.71	3.47	16.8	22.93

Fantail darter	0.01		0.02	0.13	0.09	0.03	0.20	0.16	0.02			0.66	4.58	20.61	18.23
Bowfin				0.07	0.07	0.05	0.29	0.18				0.65	12.35	10.69	13.76
Chain pickerel				0.07	0.08	0.06	0.28	0.11				0.61	11.62	9.54	29.65
White perch			0.04	0.15	0.12	0.03	0.12	0.08	0.03			0.58	13.44	15.09	16.31
Burbot				0.08	0.06	0.04	0.29	0.09				0.56	21.37	7.37	13.90
Yellow perch				0.14	0.03	0.09	0.17	0.10		0.03		0.56	10.82	9.03	17.01
Bluegill				0.13	0.10	0.05	0.13	0.12				0.52	15.18	5.11	15.39
Mean CV (%)	92	54	81	24	42	36	28	24	68	53	151	19	13	13	14

Note *ai*: *anteiso*-methyl fatty acid; ARA: Arachidonic acid; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid.

Mean Coefficients of Variation (CV) for any specific fatty acid is obtained by firstly calculating CV for each species and then taking the mean of all species.

Blank entries are those below our detection limit.

Phytanic acid, or 3, 7, 11, 15-tetramethyl hexadecanoic acid, is a plant derived polymethyl BCFA. It is widely distributed in nature and has been reported in dairy products [21] in addition to the monomethyl BCFA. More than half of fish species had phytanic acid, averaging 0.2% of total FA. Phytanic acid was found in all stream fish analyzed but was not found in most piscivorous fish living in bigger lakes. In our tabulations of BCFA we did not include phytanic acid because its post-ingestion metabolic fate, peroxisomal α-oxidation, is different than for *iso* and *anteiso* monomethyl BCFA.

Normal odd carbon numbered and trans fatty acids comprised another minor component of all fish. Margaric acid (n-17:0) was the highest odd straight chain FA with an overall mean of 0.6%, w/w. Together with n-15:0, they made up 1% of total FA and were consistently present in all measured fish samples. Additionally, small amounts of 13:0, 17:1n-8 were present in some fish. Vaccenic acid (trans-11-18:1) varied and in some cases was comparable to oleic acid (cis-9-18:1) in some fish. Trans-9-18:1 was detected in some fish at very low levels compared with either oleic acid or vaccenic acid.

BCFA Are Higher in Fish Skins

Figure 1.3 shows the percent BCFA of muscle at dorsal fin, caudal fin, belly and skin. Fish skins contain significantly more BCFA at a concentration of 1.8±0.7% of total FA. Muscle at three anatomic parts of all fishes is not significantly different, indicating a homogenous distribution of BCFA in fish muscle. Similar to fish muscle, *iso*-15:0, *anteiso*-15:0, *iso*-16:0, *iso*-17:0, *anteiso*-17:0 were also the major BCFA in fish skins. Full fatty acid profiles of fish skins are presented in the Supplement (**Table S1.4**).

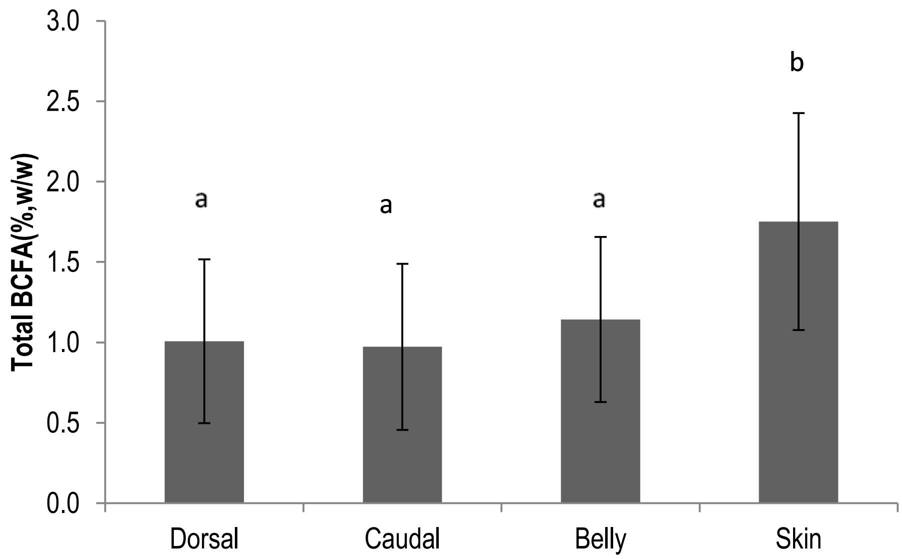
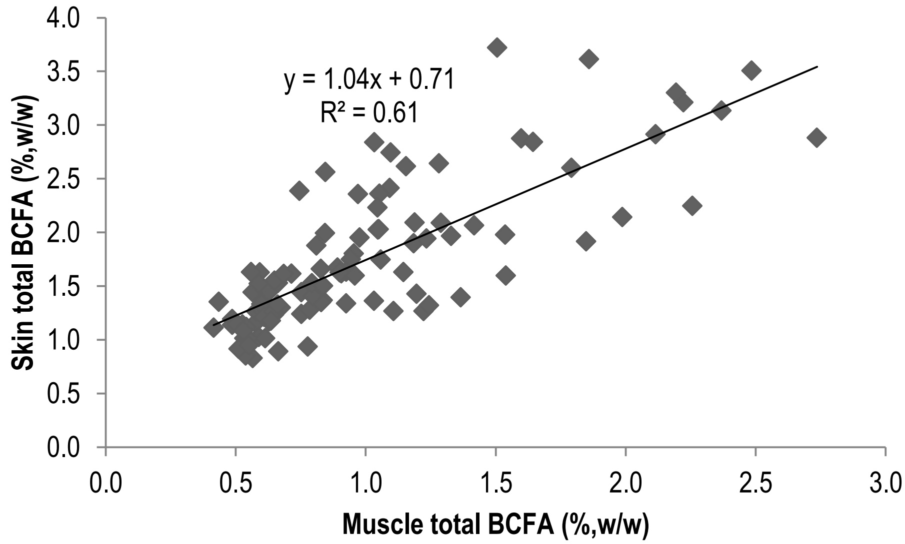


Figure 1. 3 Total BCFA (%, w/w; mean ± SD) for muscle tissues at dorsal fin, caudal fin, belly and skin

A linear regression model was used explore the relationship of BCFA in fish skin and muscle. Total skin and muscle BCFA were strongly correlated (r^2=0.6, $p<0.001$). The significant intercept of 0.7 in **figure 1.4** suggests that skin incorporate BCFA preferentially when they are available at lower levels, noting also that skin BCFA was greater than muscle BCFA in all samples. For most fish studied, *anteiso*-17:0 was among the most abundant BCFA and was very highly correlated between skin and muscle levels (r^2=0.9, $p<0.001$).



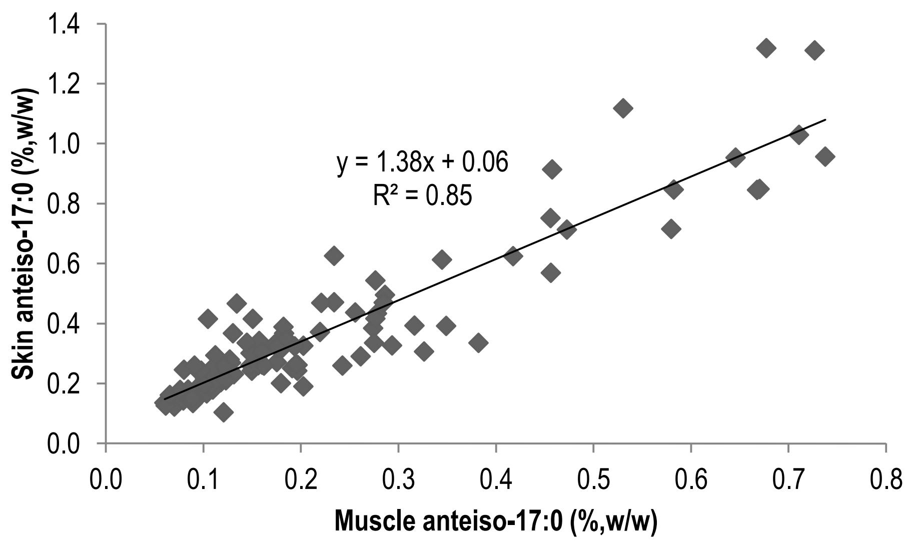


Figure 1. 4 Linear regression model between fish skin total BCFA and fish muscle total BCFA (top), $p<0.001$; fish skin anteiso-17:0 and fish muscle anteiso-17:0 (bottom), $p<0.001$

The percent weights of most fatty acids in fish skin were generally significantly different than in any part of fish muscle of the same fish. Fish skin had markedly higher concentrations of odd straight chain fatty acids, specifically n-15:0 and n-17:0. Fish skin had 10.7% higher monounsaturated fatty acids (MUFA) but 11.8% lower PUFA by weight than muscle ($p<0.001$). Fish skin was significantly lower in EPA and DHA, at 7.3±3.0% and 10.5±4.4%, respectively. Fish skin has the same amount of ARA as belly tissue but slightly lower than muscle at dorsal/caudal fins. Our paired sample t test revealed no significant difference in total saturated straight fatty acids between fish muscle and fish skin, even though both odd straight chain fatty acids and BCFA were significantly higher in fish skins.

BCFA and EPA+DHA Intake from Investigated Fish Species

While the annual world apparent consumption of fish reached 19.2 kg per capita in 2012[1], consumption in the US has always been higher than the world average. From 2009 to 2011, per capita human consumption of fish and shellfish in the US was 21.7 kg/year, corresponding to 6.6 kg (14.5 lbs) of edible seafood consumed per capita for Americans, equal to a daily intake of 18 g of seafood. The Dietary Guidelines for Americans recommend 8-12 oz of seafood per week, (32-49 g/d), about double the actual intake[22]. **Table 1.2** presents the estimated fat content of the 27 species of wild fish investigated here, along with intake of BCFA and EPA+DHA with consumption of about 70 grams or a 2.5 oz. standardized serving of any of these fish. Fat content ranged from 0.43% to 2.29% indicating that all of these wild fish would be considered lean fish compared to for instance, salmon species. Consuming one serving of these locally produced fish provides 2.5 mg to 24.2 mg of total BCFA and 107 mg to 558 mg of total EPA+DHA. This amount of BCFA would increase the estimated American BCFA daily intake of 492mg [8] by only a few milligrams, and thus add a trivial level of BCFA to the diets of those that consume dairy and beef or other ruminant foods. The greatest addition of BCFA to the diet would be from 70 g of alewife skin that could provide at most 12% fat and 207 mg BCFA, according to our data. We found no evidence of species with BCFA above a few percent in our sampling.

Table 1. 2 Total fat content of 27 fish species caught in the northeastern United States and BCFA/EPA+DHA intake from 70g fillet (Mean±STD)

	Total fat content (%)[a]	BCFA (mg) in 70g fillet	EPA+DHA (mg) in 70g fillet
Rainbow smelt	0.74±0.09	11.3±3.2	189.4±8.1
Pumpkinseed	1.67±0.48	24.2±6.0	274.5±100.8
White sucker	1.27±0.75	14.6±16.3	207.0±155.1
Lake trout	1.55±0.25	17.2±2.9	346.3±61.6
Freshwater drum	1.05±0.25	8.8±1.6	140.9±34.2
Alewife	1.09±0.28	9.0±1.7	191.3±44.4
Common shiner	0.75±0.18	6.0±2.7	112.2±8.6
White crappie	1.86±0.45	14.6±2.1	266.4±50.8
Walleye	1.14±0.31	8.6±5.5	222.6±44.9
Channel catfish	1.49±0.10	10.6±1.4	249.2±49.7
Greater redhorse	1.38±0.39	9.6±4.4	289.9±72.8
Black crappie	1.10±0.13	7.4±0.9	159.5±20.7
Smallmouth bass	0.81±0.15	5.4±2.0	138.2±38.7
Golden shiner	1.34±0.35	8.6±0.7	273.7±82.5
Slimy sculpin	0.43±0.07	2.8±1.5	107.0±18.6
Brown bullhead	1.46±0.58	9.0±2.8	249.4±121.8
Redbreast sunfish	1.48±0.79	9.0±6.7	258.7±98.2
Blacknose dace	1.08±0.24	5.4±2.8	271.7±38.5
Rock bass	2.29±0.93	11.6±5.7	558.4±223.7
Longnose dace	1.08±0.17	5.4±0.6	302.7±52.8
Fantail darter	0.53±0.10	2.5±0.4	144.4±21.1
Bowfin	1.77±0.64	8.1±2.9	304.6±88.0
Chain pickerel	1.20±0.91	5.1±4.7	329.6±247.9
White perch	0.74±0.32	3.0±1.3	164.2±71.6
Burbot	1.83±0.97	7.2±4.5	273.0±112.7
Yellow perch	0.67±0.16	2.6±0.5	122.6±23.1
Bluegill	1.05±0.38	3.8±1.3	151.6±47.2

[a]Estimated from total fatty acids without corrections for variable non-fatty acid lipid components.

Unlike dairy products, which contain similar percent of BCFA regardless of total fat content [8], various fish species exhibit a wide range of BCFA and total fat content. For example, rainbow smelt has a relatively low fat content while still providing a high amount of BCFA; on the other hand rock bass is relatively low in percent BCFA but will provide consumers with similar amount of BCFA with much higher %mass of total fat and EPA+DHA. This complicates nutritional assessment of local wild fish consumption, but gives consumers more flexibility in terms of species preference, availability, economic affordability and nutritional requirements.

BCFA levels of 27 fish species in northeastern United States ranged from 0.5%-2.2%, w/w of total FA, comparable with a few studies [9, 11, 23], lower than others [12, 24], but well below the observed 40% in flathead grey mullet reported elsewhere[10]. The high percent BCFA in the mullet could be due to its mangrove habitat and dependence on microbe-rich detritus for food. Five percent BCFA was reported in holothurians (e.g. sea cucumbers), which are considered a delicacy in Asian cuisines [25]. Holothurians feed on detritus as well and are consistent with the hypothesis that detritus feeding has a major impact on fish BCFA level. Detritus and marine sediments are enriched with BCFA due to the microbial activity, yielding similar chain length BCFA (C14-18) as are found in fish muscle [26]. Our results included more kinds of BCFA than most studies and aligned with results from Lei et al. [11], except they identified a small amount of *iso*-8:0 in one species and *iso*-10:0 in another but no *iso*-20:0 in Asian carps.

Some reports are available on the BCFA content of marine fish. Ackman presented 1.7% and 1.0% BCFA of total saturated fatty acids (SFA) in Atlantic herring (*C. harengus*) and Atlantic cod (*G. morhua*), respectively [27]. A relatively high percent of BCFA, from 2.9-7.8%,w/w of total FA, were found in various marine fishes including yellowfin tuna (*T. albacares*) [24b]. Cosper and Ackman also reported a high percent of BCFA in mummichog (*F. heteroclitus*) and Atlantic silverside (*M. menidia*) at 6.3% and 2.8%, respectively [24a].

Remarkably, EPA and DHA were from about 5% to 20% and 5% to 30% of total FA, respectively. Total EPA and DHA comprised 28% of total FA on average, indicating freshwater fish are generally very rich in n-3 PUFA, compared with about 20% and 10% EPA+DHA in salmons fed fish oil and palm oil/rapeseed oil, respectively [28]. Chain pickerel (*E. niger*) had the highest DHA of 30% and EPA of 10% while white sucker (*C. commersonii*) had 15% DHA and 9% EPA in our study. These values are very close to those of a previous study, which found a related pickerel species had 27% DHA and 10% EPA and white sucker had 17% DHA and 6% EPA [29].

Habitat and ecological factors, such as temperature and foraging mode, respectively, are known to influence fish fatty acid composition [30]. In the present work, we found that location, habitat, and foraging guild were associated with EPA but not DHA or BCFA (**Table S5**). Fish from streams had significantly greater EPA (14.5%±4.2%) than fish from lakes (8.8%±2.8%), and invertivores had greater EPA (13.7%±4.6%) than fish consuming other fish (piscivores) or with mixed diets (8.9%±2.8%). Higher EPA in stream invertivores likely originates from diatoms that have high EPA content [31] and dominate algal assemblages in the streams we sampled. These data indicate that stream fish in general may be especially good sources of EPA.

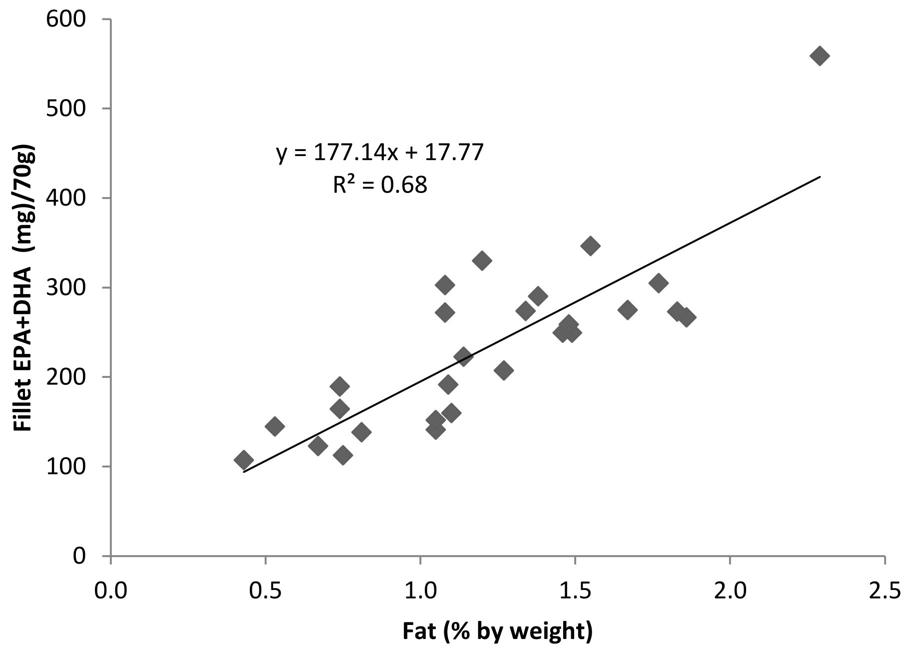
Our study complements a 2014 study that surveyed 76 species of commercially available finfish in six regions of the United States [32]. Our study identified the amount of minor BCFA, normal odd-carbon-numbered fatty acids and major PUFA across wild freshwater fish species around New York State while the previous study reported major fatty acids in wild or cultured, marine or freshwater fish. Channel catfish is the top farmed species in the United States, reported to have 8.0% fat and 31 mg EPA+DHA in 70 g fillets [32]. The wild channel catfish investigated by us had 1.5% fat and 249 mg EPA+DHA in 70 g fillets. Importantly, wild channel catfish is generally leaner but contains much higher levels of health related omega-3 fatty acids than farmed channel catfish. Most of the wild overlapping species in the two studies shared similar content of EPA+DHA, apart from white perch and lake trout which had lower EPA and DHA in our study. Total fat content, breeding status of the fish, and age may all explain differences. DHA and EPA levels in 70 g of fillet were strongly and directly correlated with total fat content (**Figure 1.5A**, r^2=0.68).

For example, white crappie (*P. annularis*) was not among those that had the highest fat percent EPA+DHA but it was a good source in terms of unit mass of fillet. Total fat content of our fish was relatively low, from 0.4-2.3%, even in the trout species. It is generally accepted that wild fish have a much lower total fat than their farmed counterparts [33].

Linoleic and linolenic acids are naturally very low in these lean wild fish, comprising only about 5% of total FA on average. Linoleic acid derived from seeds oils and grain based aquaculture feeds results in much greater tissue levels, and suppresses accumulation of omega-3 EPA and DHA [34]. Some low EPA+DHA fish such as common shiner (*L. cornutus*) and bluegill (*L. macrochirus*) had higher linoleic and linolenic acids. Although there was only a weak negative association ($r^2=0.15$, $p<0.001$) of linoleic acid and total EPA+DHA, only when linoleic acid was between 1-4% of total FA, would EPA+DHA level exceed 30%, w/w. On the other hand, when linoleic acid level was >4%, EPA+DHA level was consistently low (**Figure 1.5B**). This effect was manifest when farmed channel catfish and wild channel catfish were compared. Farmed channel catfish had 12.3% linoleic acid and 0.5% EPA+DHA, w/w in the data of Cladis et al. [32]. In stark contrast, wild channel catfish analyzed in our study had 2.6% linoleic acid and 23.8% EPA+DHA, w/w. The results point to production of channel catfish with a dramatically different nutrient profile than wild catfish. The mean linolenic acid level in our study was about half of linoleic acid and these two fatty acids were correlated

(r^2=0.45, $p<0.001$), consistent with their common terrestrial plant origin.

A



B

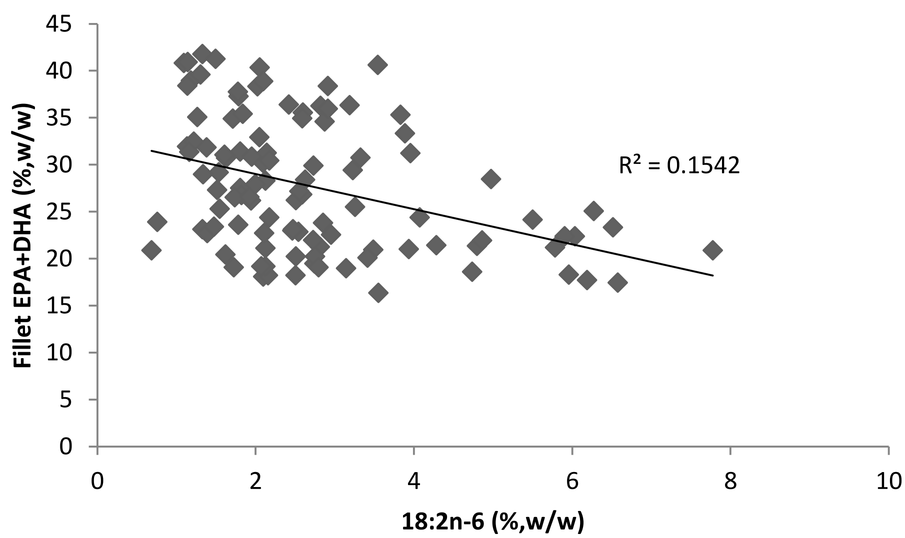


Figure 1. 5 A. Positive relationship between mass of EPA+DHA in 70 g fillet and percent fat content, $p<0.001$. B. Negative relationship between total EPA+DHA and linoleic acid (18:2n-6), $p<0.001$

n-15:0 and n-17:0 were correlated at $r^2 = 0.57$ ($p<0.001$) suggesting a common origin of odd numbered straight FA in fish or a tightly controlled elongation process from n-15:0 to n-17:0. It is generally accepted that n-15:0 and n-17:0 are biomarkers for bovine milk intake and n-15:0, at about 0.9-1.2% of total FA, is higher than n-17:0 in milk [35]. In contrast, n-17:0 was higher in our analyzed fish samples than n-15:0, in line with other reports [9, 11, 36]. Our mean level of n-17:0 was at 0.59%, w/w. A widely cited meta-analysis of intake and circulating fatty acids association with cardiovascular disease detected n-17:0 as associated with reduced risk, along with EPA, DHA, and ARA. The authors speculated that margaric acid was a biomarker of milk and dairy fat [2]. Our data suggest that n-17:0 may be an overlooked biomarker for seafood intake, from both freshwater and marine species as shown by others [9, 36b, 36c]. **Figure 1.6** presents the level of n-15:0 and n-17:0 in popular seafood in the US and Asia, plotted together with n-15:0 and n-17:0 concentrations in bovine milk. The figure demonstrates that both seafood and milk are sources of odd chain fatty acids. Moreover, the dominance of n-17:0 over n-15:0 in seafood indicates that n-17:0 may be a more specific biomarker for seafood consumption. In contrast, n-15:0 is richer in milk and may be a better indicator for milk and dairy intake.

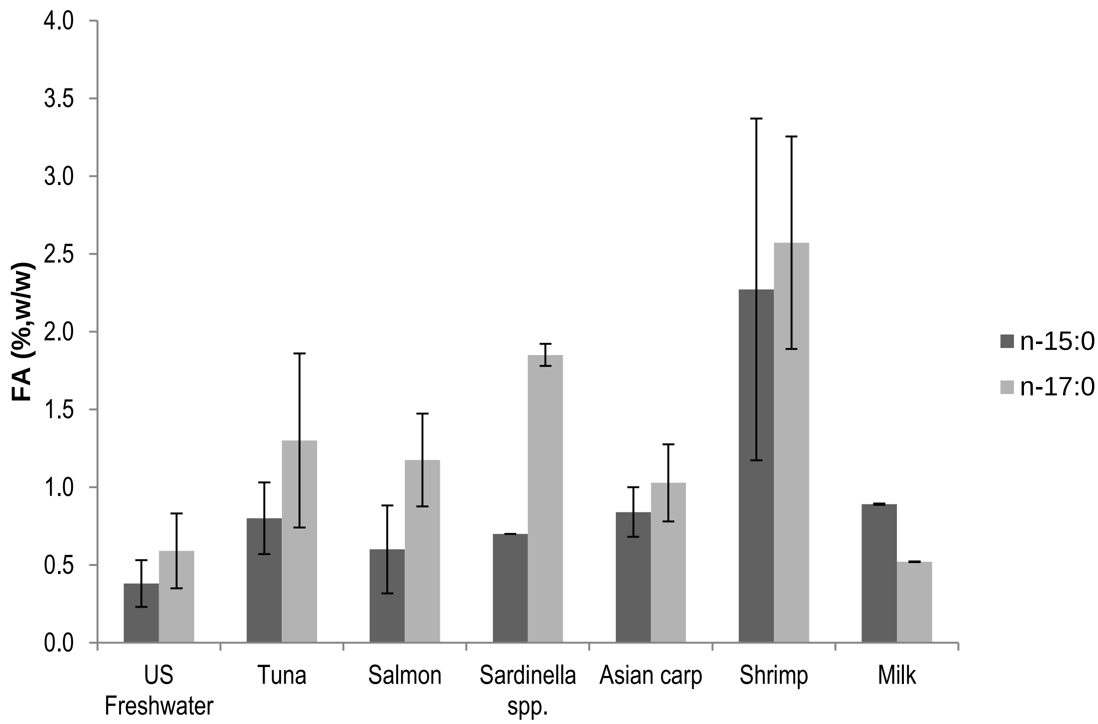


Figure 1. 6 n-15:0 and n-17:0 in US freshwater fish in the present study compared to literature data for several fishes popular in the U.S and Asian and U.S. cow's milk.

Seafood is richer in n-17:0 in all seafood species, while milk is richer in n-15:0. "Tuna" is four common tuna species reported by Roubal [36c]; "Salmon" is four salmon species reported by Gruger et al. [36b]; "Sardinella spp." includes two species reported by Njinkoue et al. [9]; "Asian carp" is four common carp species reported by Lei et al. [11]; "shrimp" includes three species reported by Bottino et al. [36a]; "Milk" is U.S. retail milk reported by O'Donnell-Megaro et al. [35]

Dairy BCFA mainly originate from bacterial fermentation in the rumen [37]. Dietary intake or synthesis in oil producing glands analogous to human sebaceous and meibomian glands is more plausible as a mechanism for fish BCFA accumulation. Besides some being piscivorous, fish eat a wide range of aquatic organisms including phytoplankton, zooplankton, macroalgae, invertebrate and its larvae. Some phytoplankton contain similar BCFA as in fish but at a higher concentration, 3-6% [38], as do various algae [39], mollusks [27, 38, 40], and shrimps [10, 40b, 40d, 41].

In conclusion, mean BCFA content was 1.0±0.5% of total FA in muscle of freshwater fish common to the northeastern United States. Fish skin had 1.8±0.7% BCFA of total FA, and linear regression showed total BCFA content in fish skin is highly correlated with BCFA concentration in fish muscle. Since a serving of fish is about 70 grams (2.5 oz.), consuming a serving of locally captured fish in the northeastern United States would provide 2.5-24.2 mg of BCFA and 107-558 mg of total EPA+DHA. Because the concentration of BCFA in fish is similar to that in ruminant foods, higher consumption of fish could contribute significant amounts of BCFA. Freshwater fish could be a major source of EPA+DHA in the human diet. Finally n-17:0 was at surprisingly high levels and may be a previously unappreciated biomarker for fish consumption.

REFERENCES

1. FAO, The State of World Fisheries and Aquaculture 2014. Rome, 2014; p 3.
2. Chowdhury, R.; Warnakula, S.; Kunutsor, S.; Crowe, F.; Ward, H. A.; Johnson, L.; Franco, O. H.; Butterworth, A. S.; Forouhi, N. G.; Thompson, S. G.; Khaw, K. T.; Mozaffarian, D.; Danesh, J.; Di Angelantonio, E., Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis. *Ann Intern Med* **2014,** *160* (6), 398-406.
3. (a) Jiao, J.; Li, Q.; Chu, J.; Zeng, W.; Yang, M.; Zhu, S., Effect of n-3 PUFA supplementation on cognitive function throughout the life span from infancy to old age: a systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr* **2014,** *100* (6), 1422-36; (b) Joffre, C.; Nadjar, A.; Lebbadi, M.; Calon, F.; Laye, S., n-3 LCPUFA improves cognition: the young, the old and the sick. *Prostaglandins Leukot Essent Fatty Acids* **2014,** *91* (1-2), 1-20; (c) Zhang, Y.; Chen, J.; Qiu, J.; Li, Y.; Wang, J.; Jiao, J., Intakes of fish and PUFAs and mild-to-severe cognitive impairment risks: a dose-response meta-analysis of 21 cohort studies. *Am J Clin Nutr* **2015**.
4. Uauy, R. D.; Birch, D. G.; Birch, E. E.; Tyson, J. E.; Hoffman, D. R., Effect of dietary omega-3 fatty acids on retinal function of very-low-birth-weight neonates. *Pediatric research* **1990,** *28* (5), 485-92.
5. U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015 – 2020 Dietary Guidelines for Americans. 8 ed.; 2015.
6. (a) Brenna, J. T.; Varamini, B.; Jensen, R. G.; Diersen-Schade, D. A.; Boettcher, J. A.; Arterburn, L. M., Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. *Am J Clin Nutr* **2007,** *85* (6), 1457-64; (b) Holman, R. T.; Johnson, S. B.; Ogburn, P. L., Deficiency of essential fatty acids and membrane fluidity during pregnancy and lactation. *Proc Natl Acad Sci U S A* **1991,** *88* (11), 4835-9.

7. Favre, H. A.; Powell, W. H., Nomenclature of organic chemistry : IUPAC recommendations and preferred names 2013. Royal Society of Chemistry: Cambridge, UK, 2013.

8. Ran-Ressler, R. R.; Bae, S.; Lawrence, P.; Wang, D. H.; Brenna, J. T., Branched-chain fatty acid content of foods and estimated intake in the USA. *The British journal of nutrition* **2014,** *112* (4), 565-72.

9. Njinkoué, J.-M.; Barnathan, G.; Miralles, J.; Gaydou, E.-M.; Samb, A., Lipids and fatty acids in muscle, liver and skin of three edible fish from the Senegalese coast: Sardinella maderensis, Sardinella aurita and Cephalopholis taeniops. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* **2002,** *131* (3), 395-402.

10. Alikunhi, N. M.; Narayanasamy, R.; Kandasamy, K., Fatty acids in an estuarine mangrove ecosystem. *Rev Biol Trop* **2010,** *58* (2), 577-87.

11. Lei, L.; Li, J.; Luo, T.; Fan, Y. W.; Zhang, B.; Ye, J.; Ye, H.; Sun, Y.; Deng, Z. Y., Predictable effects of dietary lipid sources on the fatty acids compositions of four 1-year-old wild freshwater fish from Poyang Lake. *J Agric Food Chem* **2013,** *61* (1), 210-8.

12. Rasoarahona, J. R.; Barnathan, G.; Bianchini, J. P.; Gaydou, E. M., Annual evolution of fatty acid profile from muscle lipids of the common carp (Cyprinus carpio) in Madagascar inland waters. *J Agric Food Chem* **2004,** *52* (24), 7339-44.

13. Ran-Ressler, R. R.; Khailova, L.; Arganbright, K. M.; Adkins-Rieck, C. K.; Jouni, Z. E.; Koren, O.; Ley, R. E.; Brenna, J. T.; Dvorak, B., Branched chain fatty acids reduce the incidence of necrotizing enterocolitis and alter gastrointestinal microbial ecology in a neonatal rat model. *PloS one* **2011,** *6* (12), e29032.

14. (a) Lin, T.; Yin, X.; Cai, Q.; Fan, X.; Xu, K.; Huang, L.; Luo, J.; Zheng, J.; Huang, J., 13-Methyltetradecanoic acid induces mitochondrial-mediated apoptosis in human bladder cancer cells. *Urol Oncol* **2012,** *30* (3), 339-45; (b) Wongtangtintharn, S.; Oku, H.; Iwasaki, H.; Inafuku, M.; Toda, T.; Yanagita, T.,

Incorporation of branched-chain fatty acid into cellular lipids and caspase-independent apoptosis in human breast cancer cell line, SKBR-3. *Lipids Health Dis* **2005,** *4*, 29; (c) Yang, P.; Collin, P.; Madden, T.; Chan, D.; Sweeney-Gotsch, B.; McConkey, D.; Newman, R. A., Inhibition of proliferation of PC3 cells by the branched-chain fatty acid, 12-methyltetradecanoic acid, is associated with inhibition of 5-lipoxygenase. *Prostate* **2003,** *55* (4), 281-91.

15. (a) Sağglık, S.; Alpaslan, M.; Gezgin, T.; Çetintürkc, K.; Tekinay, A.; Güven, K. C., Fatty acid composition of wild and cultivated gilthead seabream (Sparus aurata) and sea bass (Dicentrarchus labrax). *European Journal of Lipid Science and Technology* **2003,** *105* (2), 104-107; (b) Sahena, F.; Zaidul, I.; Jinap, S.; Yazid, A.; Khatib, A.; Norulaini, N., Fatty acid compositions of fish oil extracted from different parts of Indian mackerel (Rastrelliger kanagurta) using various techniques of supercritical CO 2 extraction. *Food Chemistry* **2010,** *120* (3), 879-885.

16. (a) Garces, R.; Mancha, M., One-step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues. *Anal Biochem* **1993,** *211* (1), 139-43; (b) Zhou, Y.; Nijland, M.; Miller, M.; Ford, S.; Nathanielsz, P. W.; Brenna, J. T., The influence of maternal early to mid-gestation nutrient restriction on long chain polyunsaturated fatty acids in fetal sheep. *Lipids* **2008,** *43* (6), 525-31.

17. Ran-Ressler, R. R.; Sim, D.; O'Donnell-Megaro, A. M.; Bauman, D. E.; Barbano, D. M.; Brenna, J. T., Branched chain fatty acid content of United States retail cow's milk and implications for dietary intake. *Lipids* **2011,** *46* (7), 569-76.

18. Ran-Ressler, R. R.; Lawrence, P.; Brenna, J. T., Structural characterization of saturated branched chain fatty acid methyl esters by collisional dissociation of molecular ions generated by electron ionization. *Journal of lipid research* **2012,** *53* (1), 195-203.

19. Burnham, K. P.; Anderson, D. R., Model selection and multimodel inference : a practical information-theoretic approach. 2nd ed.; Springer: New York :, 2002.

20. Simberloff, D.; Dayan, T., The guild concept and the structure of ecological communities. *Annual review of ecology and systematics* **1991,** *22*, 115-143.

21. (a) Lough, A. K., The chemistry and biochemistry of phytanic, pristanic and related acids. *Prog Chem Fats Other Lipids* **1973,** *14* (1), 1-48; (b) Vlaeminck, B.; Lourenco, M.; Bruinenberg, M.; Demeyer, D.; Fievez, V., Odd and branched chain fatty acids in rumen contents and milk of dairy cows fed forages from semi-natural grasslands. *Commun Agric Appl Biol Sci* **2004,** *69* (2), 337-40.

22. Service, N. M. F. Fisheries of the United States 2013: Current Fishery Statistics. http://www.st.nmfs.noaa.gov/Assets/commercial/fus/fus13/FUS2013.pdf (accessed May 25).

23. Ould El Kebir, M. V.; Barnathan, G.; Siau, Y.; Miralles, J.; Gaydou, E. M., Fatty acid distribution in muscle, liver, and gonads of rays (Dasyatis marmorata, Rhinobatos cemiculus, and Rhinoptera marginata) from the East Tropical Atlantic Ocean. *J Agric Food Chem* **2003,** *51* (7), 1942-7.

24. (a) Cosper, C. I.; Ackman, R. G., Occurrence of cis-9,10-methylenehexadecanoic and cis-9, 10-methyleneoctadecanoic acids in the lipids of immature and mature Fundulus heteroclitus (L.), and in roe. *Comp Biochem Physiol B* **1983,** *75* (4), 649-54; (b) Dhaneesh, K. V.; Noushad, K. M.; Kumar, T. T., Nutritional evaluation of commercially important fish species of Lakshadweep archipelago, India. *PloS one* **2012,** *7* (9), e45439.

25. Lewis, R., Fatty acid composition of some marine animals from various depths. *Journal of the Fisheries Board of Canada* **1967,** *24* (5), 1101-1115.

26. Leo, R. F.; Parker, P. L., Branched-chain Fatty acids in sediments. *Science* **1966,** *152* (3722), 649-50.

27. Ackman, R. G.; Sipos, J. C., Isolation of the saturated fatty acids of some marine lipids with particular reference to normal odd-numbered fatty acids and branched-chain fatty acids. *Comp Biochem Physiol* **1965,** *15* (4), 445-56.

28. (a) Bell, J. G.; Henderson, R. J.; Tocher, D. R.; McGhee, F.; Dick, J. R.; Porter, A.; Smullen, R. P.; Sargent, J. R., Substituting fish oil with crude palm oil in the diet of Atlantic salmon (Salmo salar) affects muscle fatty acid composition and hepatic fatty acid metabolism. *J Nutr* **2002,** *132* (2), 222-30; (b) Bell, J. G.; McEvoy, J.; Tocher, D. R.; McGhee, F.; Campbell, P. J.; Sargent, J. R., Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (Salmo salar) affects tissue lipid compositions and hepatocyte fatty acid metabolism. *J Nutr* **2001,** *131* (5), 1535-43.

29. Moghadasian, M. H.; Moghadasian, P.; Le, K.; Hydamaka, A.; Zahradka, P., Lipid Analyses of Four Types of Fish From Manitoba Lakes. *E Chronicon Nutrition* **2015,** *1* (2), 41-48.

30. Ahlgren, G.; Blomqvist, P.; Boberg, M.; Gustafsson, I. B., Fatty acid content of the dorsal muscle—an indicator of fat quality in freshwater fish. *Journal of Fish Biology* **1994,** *45* (1), 131-157.

31. Twining, C. W.; Brenna, J. T.; Hairston, N. G.; Flecker, A. S., Highly unsaturated fatty acids in nature: what we know and what we need to learn. *Oikos* **2015**.

32. Cladis, D. P.; Kleiner, A. C.; Freiser, H. H.; Santerre, C. R., Fatty acid profiles of commercially available finfish fillets in the United States. *Lipids* **2014,** *49* (10), 1005-18.

33. (a) Ackman, R. G.; Takeuchi, T., Comparison of fatty acids and lipids of smolting hatchery-fed and wild Atlantic salmon Salmo salar. *Lipids* **1986,** *21* (2), 117-20; (b) Karapanagiotidis, I. T.; Bell, M. V.; Little, D. C.; Yakupitiyage, A.; Rakshit, S. K., Polyunsaturated fatty acid content of wild and farmed tilapias in Thailand: effect of aquaculture practices and implications for human nutrition. *J Agric Food Chem* **2006,** *54* (12), 4304-10.

34. Gibson, R. A.; Muhlhausler, B.; Makrides, M., Conversion of linoleic acid and alpha-linolenic acid to long-chain polyunsaturated fatty acids (LCPUFAs), with a focus on pregnancy, lactation and the first 2 years of life. *Matern Child Nutr* **2011,** *7 Suppl 2*, 17-26.

35. O'Donnell-Megaro, A. M.; Barbano, D. M.; Bauman, D. E., Survey of the fatty acid composition of retail milk in the United States including regional and seasonal variations. *J Dairy Sci* **2011,** *94* (1), 59-65.

36. (a) Bottino, N. R.; Gennity, J.; Lilly, M. L.; Simmons, E.; Finne, G., Seasonal and nutritional effects on the fatty acids of three species of shrimp, Penaeus setiferus, P. aztecus and P. duorarum. *Aquaculture* **1980,** *19* (2), 139-148; (b) Gruger, E. H.; Nelson, R.; Stansby, M. E., Fatty acid composition of oils from 21 species of marine fish, freshwater fish and shellfish. *Journal of the American Oil Chemists Society* **1964,** *41* (10), 662-667; (c) Roubal, W. T., Tuna fatty acids: II. Investigation of the composition of raw and processed domestic tuna. *Journal of the American Oil Chemists Society* **1963,** *40* (6), 215-218.

37. Keeney, M.; Katz, I.; Allison, M., On the probable origin of some milk fat acids in rumen microbial lipids. *Journal of the American Oil Chemists Society* **1962,** *39* (4), 198-201.

38. Budge, S.; Parrish, C.; Mckenzie, C., Fatty acid composition of phytoplankton, settling particulate matter and sediments at a sheltered bivalve aquaculture site. *Marine Chemistry* **2001,** *76* (4), 285-303.

39. Heiba, H. I.; Al-Easa, H. S.; Rizk, A. F., Fatty acid composition of twelve algae from the coastal zones of Qatar. *Plant Foods Hum Nutr* **1997,** *51* (1), 27-34.

40. (a) Meziane, T.; Tsuchiya, M., Fatty acids as tracers of organic matter in the sediment and food web of a mangrove/intertidal flat ecosystem, Okinawa, Japan. *Marine Ecology Progress Series* **2000,** *200*, 49-57; (b) Perry, G.; Volkman, J.; Johns, R.; Bavor, H., Fatty acids of bacterial origin in contemporary marine sediments. *Geochimica et Cosmochimica Acta* **1979,** *43*

(11), 1715-1725; (c) Zhukova, N. V.; Kharlamenko, V. I.; Svetashev, V. I.; Rodionov, I. A., Fatty acids as markers of bacterial symbionts of marine bivalve molluscs. *Journal of Experimental Marine Biology and Ecology* **1992,** *162* (2), 253-263; (d) Calado, R.; Rosa, R.; Morais, S.; Nunes, M. L.; Narciso, L., Growth, survival, lipid and fatty acid profile of juvenile monaco shrimp Lysmata seticaudata fed on different diets. *Aquaculture research* **2005,** *36* (5), 493-504.

41. (a) Calado, R.; Figueiredo, J.; Rosa, R.; Nunes, M.; Narciso, L., Effects of temperature, density, and diet on development, survival, settlement synchronism, and fatty acid profile of the ornamental shrimp Lysmata seticaudata. *Aquaculture* **2005,** *245* (1), 221-237; (b) Fricke, H.; Gercken, G.; Schreiber, W.; Oehlenschläger, J., Lipid, sterol and fatty acid composition of Antarctic krill (Euphausia superba Dana). *Lipids* **1984,** *19* (11), 821-827.

CHAPTER 2

The Asian fermented foods nātto and shrimp paste contain high levels of branched chain fatty acids*

Abstract

The predominant source of branched chain fatty acids (BCFA, or branched fat) intake in western countries is from dairy products, beef and other ruminant products, supporting a mean intake of 500 mg per day among Americans. Breast milk from Chinese mothers contains BCFA though Asians consume relatively little dairy products. We sought to identify food BCFA sources in Asian countries, and hypothesized that fermentation may add significant BCFA to foods that otherwise have little. We selected the popular Asian foods nātto and kimchi because their fermenting microorganisms are known to produce BCFA. Shrimp paste was included because seafood is a potential source of BCFA and its complex fermentation process could further increase the level. We also included miso and douchi, higher fat foods that may contribute BCFA even if of low BCFA concentration. We confirmed that high levels of fatty acid ethyl esters (FAEE) were present in commercial miso products and unexpectedly found a significant amount (6%) of FAEE in homemade kimchi. BCFA concentrations were 1.71±0.17%, 3.18 ± 0.14%, 0.37±0.06%, 0.64±0.08% and 0.08±0.01% of total fatty acids in nātto, shrimp paste, miso, homemade kimchi and douchi, respectively. The major BCFA in nātto are *iso*-14:0, *iso*-15:0, *anteiso*-15:0, *iso*-16:0, *iso*-17:0 and *anteiso*-17:0, substantially

recapitulating the BCFA profile of fluid milk. Consuming one typical serving (90 g) of nātto provides 117 mg of BCFA. Nātto and shrimp paste are the first fermented foods identified with BCFA approaching or exceeding that of milkfat (2.0% BCFA). Because of its substantial fat content, nātto makes a significant contribution of BCFA to the diet in habitual consumers.

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Introduction

Fermented foods play important roles in the diets and culture of Asian countries. Some varieties are as common as yogurt in the western world. For instance, fermented vegetables known as kimchi is a daily part of Korean diets, and mold fermented soy miso is a common food in Japan. Douchi is a fermented black soybean product considered an indispensable ingredient in Chinese kitchens across the country. Nātto is a traditional Japanese food made from fermented cooked soybeans, consumed by many Japanese weekly. Some specific data on chronic disease prevention is available for some foods. For instance, nātto intake is associated with less bone loss in Japanese postmenopausal women[1]. There is also non-vegetarian version in most parts of Asia, e.g. shrimp paste which uses mostly *Acetes* species as the fermentation substrates. While dairy consumption in Asian countries is low, these fermented oriental foods contribute protein, fat and vitamins in a traditional cereal based diet.

In terms of lipid metabolism, *Bacillus subtilis* biosynthesizes a great amount of branched chain fatty acids (BCFA) as its membrane component at about 95%,w/w of total fatty acids[2]. The fact that nātto is primarily fermented by *B. subtilis* leads to our speculation that nātto may have a unique lipid profile due to the interactions with *B. subtilis.* Kimchi is fermented by lactic acid bacteria[3] which are another group of BCFA producers[4]. Fermented foods may also have distinct fatty acid properties from the unfermented raw food, specifically a high concentration of fatty acid ethyl esters (FAEE) as are found in miso[5].

BCFA are mostly saturated fatty acids with a terminal propan-2-yl (isopropyl) group (iso) or butan-2-yl (sec-butyl) group (anteiso)[6]. Food BCFA originate primarily from bacterial action and a typical rumen fermentation in the USA results in a 2% BCFA in milk fat profile,[7] though milks of some animals can exceed 3%[8]. Together with beef, BCFA daily intake for an average American is about 500 mg[9], greater than other bioactive fatty acids such as omega-3 EPA and DHA averaging 100 mg for Americans[10]. High BCFA intake from dairy and beef products may improve gut health. Early evidence indicates that BCFA alter nascent microbiota and reduce the incidence of necrotizing enterocolitis in a neonatal rat study[11], and reduce pro-inflammatory markers in a human intestinal cell line[12].

In Asian countries where dairy consumption is relatively low, intake of potential health-promoting BCFA is expected to be low. However, a recent study found a comparable amount of BCFA in the breast milk donated by Chinese mothers and American mothers. We speculate that other Asian foods may be rich in BCFA. The goal of this study is to determine the BCFA composition of popular fermented Asian foods that are fermented by BCFA producing microorganisms and/or high in fat content. Nātto and kimchi were chosen mainly because they are fermented by BCFA producing microorganisms while miso and douchi are high in fat that could contribute significant amount of BCFA even concentration is low. Although it is unclear if *Acetes* species are already rich in BCFA, as seen in some true krill such as Antarctic krill (*Euphausia superba*)[13], shrimp paste undergoes complex bacterial fermentation that could also contribute to BCFA content.

Methods

Sampling

Kimchi was homemade by a traditional recipe, including napa cabbage, red pepper, carrot, salt and a small amount of shrimp paste. Shrimp paste (Lee Kum Kee International Holdings Ltd., Hong Kong, China) was purchased on Amazon. Miso (Aka miso, Kabuto Inc.) was purchased from a local supermarket in Ithaca, NY (Distributed by Rhee Bros., Inc., 7461 Coca Cola

Drive, Hanover, MD, USA) and originated in Japan. Two different flavors of nātto were purchased from a local Asian grocery in Ithaca, NY (Mizkan Co., Ltd., 2-6, Nakamura-cho, Handa-shi, Aichi-ken, Japan). Douchi was obtained from a local supermarket in Ithaca, NY (Koon Chun, Inc., Hong Kong, China). Dried shrimps (*Acetes* species) were purchased from a local Asian grocery in Ithaca, NY (Eastern Oceanic Enterprise, Brooklyn, NY). Aliquots of shrimp paste, dried shrimp, miso, kimchi and douchi were processed and analyzed in duplicate. All samples were analyzed as soon as practical after purchase and transport to the lab.

Fatty Acid Analysis

Samples (50-250mg) of each food were extracted, saponified and methylated according to a modified one-step hydrolysis procedure described previously by others[14]. Methylated fatty acids were then analyzed with a Hewlett Packard 5890 Gas Chromatograph (GC) equipped with a split/splitless injector run in splitless mode at 250 °C, and with the flame ionization detector (FID) set at 270°C. A BPX-70 column (25 m x 0.22 mm x 0.25 lm, SGE, Austin, TX) was used with hydrogen as the carrier gas. The oven temperature program was initially 80 °C for one minute, increased by 30°C per minute to 170°C and held for 2 min, then increased by 10°C per minute until a final temperature of 240 °C which was held for 1 min. An equal weight FAME mixture (GLC462; Nu-Chek Prep, Elysian, MN) was used to calculate response factors. BCFA were identified by electron ionization mass spectrometry (EIMS) and EIMS/MS described previously[15]. Several pure BCFA were also used as reference standards: *iso*-14:0, *anteiso*-15:0; *iso*-16:0, *anteiso*-17:0, *iso*-18:0 and *iso*-20:0 (Larodan Fine Chemicals AB, Malmo, Sweden).

Results and Discussion

Nātto BCFA concentrations were 1.71 ± 0.17%,w/w of total fatty acids (mean ± SD) and fermented shrimp paste had the highest concentration of BCFA at 3.18 ± 0.14%, exceeding the proportion of 2% BCFA in fluid milk in the USA[7]. BCFA were 0.37 ± 0.06%, 0.64 ± 0.08% and 0.08 ± 0.01% in miso, homemade kimchi and douchi, respectively. *iso*-14:0, *iso*-15:0, *anteiso*-15:0, *iso*-16:0, *iso*-17:0, *anteiso*-17:0 and *iso*-18:0 were all detected in kimchi and nātto except *iso*-18:0 was below detection limit in nātto. Shrimp paste had all the aforementioned BCFA except for *iso*-14:0. In addition, it also contained *iso*-20:0 and *iso*-24:0. *Only iso*-15:0, *iso*-16:0, *anteiso*-17:0 and *iso*-18:0 were found in miso. Low concentrations of *iso*-15:0, *anteiso*-15:0, *iso*-16:0, *iso*-17:0 and *anteiso*-17:0 were found in douchi. *iso*-17:0 alone approached 1% in shrimp paste and *anteiso*-15:0 was highest at 0.56% in nātto. However, *iso*-15:0 and *anteiso*-17:0 were highest in miso and kimchi, respectively. Despite *anteiso*-15:0 alone made up a third of BCFA in nātto, total *iso*-BCFA were slightly higher than *anteiso*-BCFA in nātto and this trend applied to kimchi, miso and douchi as well. In the case of shrimp paste, *iso*-BCFA were much higher than *anteiso*-BCFA (**Table 2.1**).

Table 2. 1 BCFA concentrations (wt% of total fatty acids) in nātto, shrimp paste, miso, kimchi and douchi (mean±SD)

BCFA (wt%)	Nātto	Shrimp paste	Miso	Kimchi[a]	Douchi
iso-14:0	0.23±0.05		<0.003	0.06±0.01	<0.003
iso-15:0	0.26±0.01	0.27±0.04	0.20±0.00	0.04±0.01	0.01±0.01
anteiso-15:0	0.56±0.02	0.09±0.04	<0.003	0.07±0.00	0.02±0.01
iso-16:0	0.33±0.04	0.33±0.06	0.04±0.00	0.12±0.01	0.01±0.00
iso-17:0	0.19±0.06	0.96±0.20	<0.003	0.08±0.00	0.02±0.00
anteiso-17:0	0.14±0.01	0.26±0.03	0.10±0.03	0.21±0.02	0.01±0.00
iso-18:0	<0.003	0.37±0.10	0.04±0.02	0.06±0.02	<0.003
iso-20:0		0.24±0.18			
iso-24:0		0.66±0.03			
Total *iso*	1.01±0.16	2.83±0.60	0.28±0.02	0.36±0.05	0.04±0.01
Total *anteiso*	0.7±0.03	0.35±0.07	0.10±0.03	0.28±0.02	0.03±0.01
Total BCFA	1.71±0.17	3.18±0.14	0.37±0.06	0.64±0.08	0.08±0.01

a The addition of shrimp paste into homemade kimchi is mainly for flavor and such small addition made negligible contribution to BCFA concentration in kimchi based on our calculations.

The nātto products contain about 8% fat according to the package. Using this figure, one gram of nātto would provide 1.3 ± 0.13 mg BCFA, double that of the 0.67 ± 0.05 mg BCFA in 1 g of whole milk. Shrimp paste is low in fat but its high concentration makes it also a good source of BCFA at 0.55±0.03 mg per gram of the food. Miso is lower at 0.23 ± 0.04 mg BCFA and kimchi at 0.03 ± 0.00 mg BCFA per gram of the foods. One half cup serving of nātto is about 90 g, and one serving of whole milk is about one cup (244g). When serving size is taken into account, a serving of nātto and whole milk provide comparable amount of BCFA (>100 mg/serving), which exceeds the daily intake of EPA+DHA for Americans. Shrimp paste, kimchi, miso and douchi provide small amounts of BCFA (~1-9 mg/serving, **Table 2.2**). Not surprisingly linoleic acid comprises more than 50% of nātto total fatty acids, followed by palmitic acid, oleic acid, alpha-linolenic acid and stearic acid (supplementary table 1). All other fatty acids were below 1%. Saturated fatty acids comprise about a fourth of total fatty acids by weight. The two polyunsaturated fatty acids (PUFA), linoleic acid and alpha-linolenic acid, in nātto represent about 63.5% of total fatty acids, all consistent with the fatty acid profile of soybeans.

Table 2. 2 Branched chain fatty acids in nātto, shrimp paste, miso, kimchi and douchi in comparison with milk

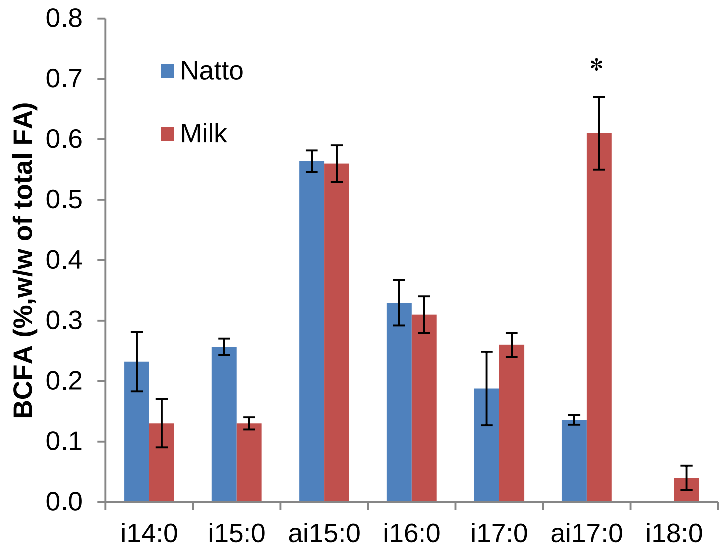
Fermented Food	Fat (%)[a]	BCFA (mg fatty acid/ g sample)	BCFA (mg fatty acid/ serving)
Milk	3.25	0.67±0.05[b]	(244 g) 158
Nātto	7.78	1.33±0.13	(90 g) 117
Shrimp paste	1.74	0.55±0.03	(16 g) 8.8
Miso	6.25	0.23±0.04	(16 g) 3.7
Kimchi	0.50	0.03±0.00	(90 g) 2.9
Douchi	9.42	0.07±0.01	(16 g) 1.2

a. Percent fat of nātto and miso was obtained from product information and shrimp paste, kimchi and douchi from the addition of an internal standard.

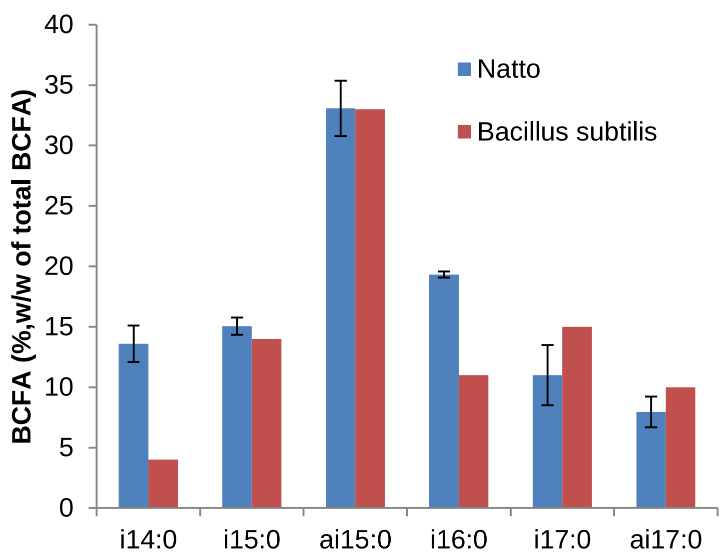
b. Milk BCFA concentration is calculated from reporting by Ran-Ressler[7]

The BCFA profile of nātto shows a remarkable similarity with milk fat (**Figure 2.1a**). While bacterial contribution to milk fat is the net result of the complex rumen microbiota, nātto is a product characteristic of *B. subtilis* fermentation. The comparison of nātto fatty acid profile with the compositional fatty acid profile of *B. subtilis* shows that they have the identical chain length for BCFA[2]. When each BCFA is expressed as percent weight of total BCFA, *anteiso*-15:0 is the predominant BCFA at about 33% for both nātto and its fermenting bacteria. The remaining *iso-15:0, iso-17:0* and *anteiso-17:0* are in the same proportions while *iso-14:0* and *iso-16:0* have some variability (**Figure 2.1b**). This variability may be due to the difference of substrates, specifically soybean for nātto and medium for *B. subtilis* incubation. Considering the above findings and low BCFA in mold-fermented miso, we are the first to conclude that BCFA producing bacteria can significantly modify and increase the BCFA content of a fermented food, as seen with *B. subtilis* contributing its compositional BCFA to nātto.

a



b

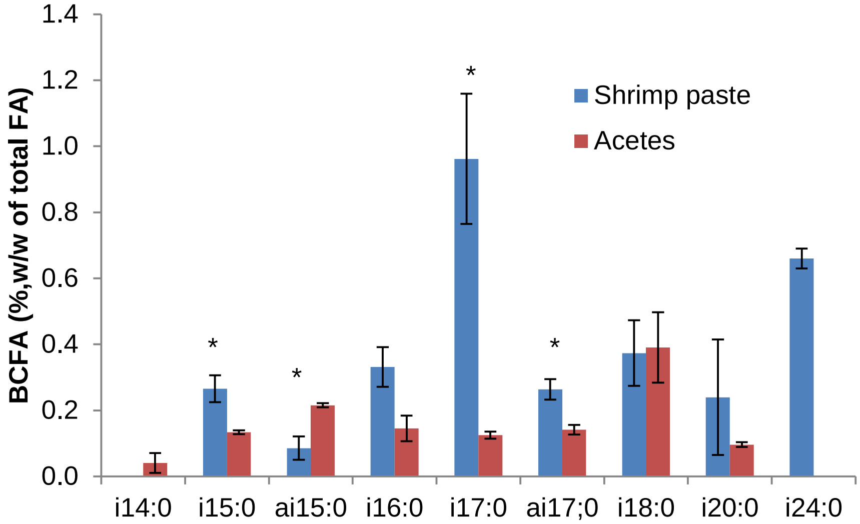


* p<0.05

Figure 2. 1 Nātto BCFA content is similar to milk[7] except for *anteiso*-17:0 and significantly resemble its fermenting agent, *B. subtilis*[2]

Shrimp paste has a notably high concentration of BCFA at 3.18 ± 0.14%, w/w of total fatty acids. This BCFA enrichment could be accounted for by the BCFA naturally contained in *Acetes* species and the fermentation process. The fatty acid profile of dried *Acetes* was determined and the BCFA proportion was presented with shrimp paste, its fermented product in **Figure 2.2**. Dried *Acetes* has 1.29 ± 0.10% total BCFA of total fatty acids, considerably lower than shrimp paste. Compared with dried *Acetes,* shrimp paste is significantly higher in *iso*-15:0, *iso*-17:0, *anteiso*-17:0 and lower in *anteiso*-15:0. Two very long chain BCFA, *iso*-20:0 and *iso*-24:0 were found in shrimp paste and *iso*-20:0 in dried *Acetes*. The fermentation process of shrimp paste is not well understood but there are a bunch of studies on its fermenting agents. Surono et al. reported that the most abundant microflora in "Terasi" shrimp paste was in the order of *Bacillus, Pseudomonas, Micrococcus, Kurthia* and *Sporolactobacillus*[16]. Other lactic acid bacteria such as halophilic *tetragenococcus halophilus* and *T. muriaticus* were also found in "Terasi" shrimp paste[17]. Species in the genera of *Bacillus, Pseudomonas, Micrococcus* and many lactic acid bacteria are well known to incorporate extremely high amounts of BCFA in their membranes[4]. Two strains of *Lentibacillus kapialis* were directly isolated from a fermented shrimp paste in Thailand and found to contain 98% cellular BCFA[18]. These evidences showed that bacterial fermentation further enriched the BCFA content in small shrimps. Therefore, we here discovered and first demonstrated, as seen in shrimp paste that fermentation could result in a product with higher concentration of BCFA

(~3%) than dairy foods.



* p<0.05

Figure 2. 2 BCFA comparison of fermented shrimp paste and a commercially available dried shrimp (Acetes species)

The levels of EPA and DHA in shrimp paste and its original dried small shrimp are also remarkable. The total EPA and DHA in shrimp paste and dried small shrimp are 28% and 27% of total fatty acids, the same level as the averaging 27 freshwater fishes in the northeastern US[19], and higher than farmed salmon fed either fish oil or palm oil/rapeseed oil[20]. This makes fermented shrimp paste and dried small shrimp qualified as shelf-stable omega-3 fatty acid replenishment especially for those who don't have access to live, frozen seafood or omega-3 supplements. Both the shrimp paste and dried small shrimp are very low in omega-6 fatty acids, for example linoleic acid at 1.5% and arachidonic acid at 3-4%. People frequently consuming these foods are expected to maintain lower omega-6/omega-3 ratio. Despite shrimp paste is enriched in BCFA, all the major fatty acids of the two foods are in the same concentration.

FAEE are a lipid class that is normally at very low concentration in living tissue and thus raw foods. In miso, we here found 39.49 ± 0.33% total FAEE as a fraction of total fatty acids, in line with Yamabe et al. who reported 35% total FAEE of total lipids in maturated miso[5]. Linoleic acid (18:2n-6) ethyl ester is the highest at 21.18 ± 0.31% of total fatty acids, followed by 16:0, 18:1n-9 and 18:3n-3 ethyl ester, again consistent with the previous report.[5] In addition to those ethyl esters reported previously, we also found 0.72 ± 0.00% and 0.10 ± 0.01% 22:0 and 20:0 ethyl esters in miso. Based on the fat content of this miso product, total FAEE content is estimated at 24.68 ± 0.20 mg/g of sample.

PUFAs in miso are 33.53 ± 0.01% of total fatty acids with linoleic acid dominating (**Table S2.3**). Saturated fatty acids comprise a total of 13.60 ± 0.33% and palmitic acid is the highest at 8.57 ± 0.14%.

Incidental to this BCFA study we detected FAEE as a significant components of kimchi, 6.6 ± 0.10% of total fatty acids, which has not been reported previously to our knowledge. Similar to miso, linoleic acid ethyl ester is at the highest concentration, 3.69 ± 0.03%. Palmitic acid comprises 21.62 ± 0.07% of total fatty acids, the highest among saturates. Linoleic acid and alpha-linolenic acid dominates the polyunsaturated portion. Surprisingly we found small quantities of long chain PUFA in kimchi, 0.91 ± 0.05% EPA+DHA, possibly a significant quantity for those consuming kimchi daily and not obtaining these fatty acids from other sources. Notably, our kimchi was a properly fermented homemade product to avoid commercial products that are not fermented but instead acidified with vinegar. Such pickled kimchi is not expected to have fatty acids or lipid classes (e.g. no FAEE) different from the cabbage from which it is made. Indeed the presence of FAEE may be a simple means to test whether a product labeled as kimchi has been fermented.

Douchi has low BCFA content compared with nātto. Theoretically, there is little difference between their fermentation substrates, black soybean and white soybean. The discrepancy in BCFA content would come from the fermentation process. While nātto is fermented by *B. subtilis,* douchi has two different fermentation types, which involve *Aspergillus oryzae/Rhizopus oryzae* or *B. subtilis,* respectively. Our purchased product falls into the first category. A Chinese publication reports that most of the douchi products are fermented by the aforementioned molds and presented as a dry form[21]. Bacteria fermented douchi is usually produced on a small scale or homemade and we were not able to obtain genuine samples of this form of douchi. To the best of our knowledge, molds do not produce BCFA as many bacterial genera do. This could well justify why “dry” douchi and tempeh[9] are low in BCFA. Further research can compare the fatty acid composition of nātto and bacterial fermented “wet” douchi since the same bacteria species is involved in their production.

Fermented foods have numerous benefits compared with non-fermented counterparts, most notably that fermented foods are better preserved than non-fermented counterparts. Nutritional benefits have long been known. For example, the protein digestibility of nātto is higher than for raw soybeans which have a trypsin inhibitor. It is also remarkable that fermented shrimp paste still maintains an appreciable level of EPA and DHA. In recent years, the probiotic and prebiotic properties of foods have been more clearly recognized. Nātto is fermented by *Bacillus subtilis* which was found to improve the growth and survival of pigs[22] and various fish and shrimp[23]. The increasing appreciation of the benefits of fermented foods could lead to a rise in their consumption and the intake of BCFA. In fact, BCFA intake is already high for diets with habitual consumption of nātto, in an amount greater than the 100 mg EPA and DHA that an American would consume in a day on average. Our data show that branched fats are more widely distributed in human diets than previously known and support further research into the health effects of these little studied food components.

REFERENCES

1. Ikeda, Y.; Iki, M.; Morita, A.; Kajita, E.; Kagamimori, S.; Kagawa, Y.; Yoneshima, H., Intake of fermented soybeans, natto, is associated with reduced bone loss in postmenopausal women: Japanese Population-Based Osteoporosis (JPOS) Study. *J Nutr* **2006,** *136* (5), 1323-8.
2. Kaneda, T., Fatty acids of the genus Bacillus: an example of branched-chain preference. *Bacteriol Rev* **1977,** *41* (2), 391-418.
3. Lee, C.-H., Lactic acid fermented foods and their benefits in Asia. *Food control* **1997,** *8* (5), 259-269.
4. Kaneda, T., Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. *Microbiological reviews* **1991,** *55* (2), 288-302.
5. Yamabe, S.; Kaneko, K.; Inoue, H.; Takita, T., Maturation of fermented rice-koji miso can be monitored by an increase in fatty acid ethyl ester. *Biosci Biotechnol Biochem* **2004,** *68* (1), 250-2.
6. Favre, H. A.; Powell, W. H., Nomenclature of organic chemistry : IUPAC recommendations and preferred names 2013. Royal Society of Chemistry: Cambridge, UK, 2013.
7. Ran-Ressler, R. R.; Sim, D.; O'Donnell-Megaro, A. M.; Bauman, D. E.; Barbano, D. M.; Brenna, J. T., Branched chain fatty acid content of United States retail cow's milk and implications for dietary intake. *Lipids* **2011,** *46* (7), 569-76.
8. Or-Rashid, M. M.; Odongo, N. E.; Subedi, B.; Karki, P.; McBride, B. W., Fatty acid composition of yak (Bos grunniens) cheese including conjugated linoleic acid and trans-18:1 fatty acids. *J Agric Food Chem* **2008,** *56* (5), 1654-60.
9. Ran-Ressler, R. R.; Bae, S.; Lawrence, P.; Wang, D. H.; Brenna, J. T., Branched-chain fatty acid content of foods and estimated intake in the USA. *The British journal of nutrition* **2014,** *112* (4), 565-72.

10. (a) Brenna, J. T.; Lapillonne, A., Background paper on fat and fatty acid requirements during pregnancy and lactation. *Ann Nutr Metab* **2009,** *55* (1-3), 97-122; (b) Ervin, R. B.; Wright, J. D.; Wang, C. Y.; Kennedy-Stephenson, J., Dietary intake of fats and fatty acids for the United States population: 1999-2000. *Adv Data* **2004,** (348), 1-6.

11. Ran-Ressler, R. R.; Khailova, L.; Arganbright, K. M.; Adkins-Rieck, C. K.; Jouni, Z. E.; Koren, O.; Ley, R. E.; Brenna, J. T.; Dvorak, B., Branched chain fatty acids reduce the incidence of necrotizing enterocolitis and alter gastrointestinal microbial ecology in a neonatal rat model. *PloS one* **2011,** *6* (12), e29032.

12. Yan, Y.; Wang, Z.; Greenwald, J.; Kothapalli, K. S.; Park, H. G.; Liu, R.; Mendralla, E.; Lawrence, P.; Wang, X.; Brenna, J. T., BCFA suppresses LPS induced IL-8 mRNA expression in human intestinal epithelial cells. *Prostaglandins Leukot Essent Fatty Acids* **2017,** *116*, 27-31.

13. Fricke, H.; Gercken, G.; Schreiber, W.; Oehlenschlager, J., Lipid, Sterol and Fatty-Acid Composition of Antarctic Krill (Euphausia-Superba Dana). *Lipids* **1984,** *19* (11), 821-827.

14. (a) Garces, R.; Mancha, M., One-step lipid extraction and fatty acid methyl esters preparation from fresh plant tissues. *Anal Biochem* **1993,** *211* (1), 139-43; (b) Zhou, Y.; Nijland, M.; Miller, M.; Ford, S.; Nathanielsz, P. W.; Brenna, J. T., The influence of maternal early to mid-gestation nutrient restriction on long chain polyunsaturated fatty acids in fetal sheep. *Lipids* **2008,** *43* (6), 525-31.

15. Ran-Ressler, R. R.; Lawrence, P.; Brenna, J. T., Structural characterization of saturated branched chain fatty acid methyl esters by collisional dissociation of molecular ions generated by electron ionization. *Journal of lipid research* **2012,** *53* (1), 195-203.

16. Surono, I. S.; Hosono, A., Chemical and aerobic bacterial composition of “Terasi”, a traditional fermented product from Indonesia. *Food Hygiene and Safety Science (Shokuhin Eiseigaku Zasshi)* **1994,** *35* (3), 299-304_1.

17. Kobayashi, T.; Kajiwara, M.; Wahyuni, M.; Kitakado, T.; Hamada-Sato, N.; Imada, C.; Watanabe, E., Isolation and characterization of halophilic lactic acid bacteria isolated from "terasi" shrimp paste: a traditional fermented seafood product in Indonesia. *J Gen Appl Microbiol* **2003,** *49* (5), 279-86.
18. Pakdeeto, A.; Tanasupawat, S.; Thawai, C.; Moonmangmee, S.; Kudo, T.; Itoh, T., Lentibacillus kapialis sp. nov., from fermented shrimp paste in Thailand. *International journal of systematic and evolutionary microbiology* **2007,** *57* (2), 364-369.
19. Wang, D. H.; Jackson, J. R.; Twining, C.; Rudstam, L. G.; Zollweg-Horan, E.; Kraft, C.; Lawrence, P.; Kothapalli, K.; Wang, Z.; Brenna, J. T., Saturated Branched Chain, Normal Odd-Carbon-Numbered, and n-3 (Omega-3) Polyunsaturated Fatty Acids in Freshwater Fish in the Northeastern United States. *J Agric Food Chem* **2016**.
20. (a) Bell, J. G.; Henderson, R. J.; Tocher, D. R.; McGhee, F.; Dick, J. R.; Porter, A.; Smullen, R. P.; Sargent, J. R., Substituting fish oil with crude palm oil in the diet of Atlantic salmon (Salmo salar) affects muscle fatty acid composition and hepatic fatty acid metabolism. *J Nutr* **2002,** *132* (2), 222-30; (b) Bell, J. G.; McEvoy, J.; Tocher, D. R.; McGhee, F.; Campbell, P. J.; Sargent, J. R., Replacement of fish oil with rapeseed oil in diets of Atlantic salmon (Salmo salar) affects tissue lipid compositions and hepatocyte fatty acid metabolism. *J Nutr* **2001,** *131* (5), 1535-43.
21. 蒋立文, 发酵豆豉的研究进展. *食品安全质量检测学报* **2013,** (6).
22. Guo, X.; Li, D.; Lu, W.; Piao, X.; Chen, X., Screening of Bacillus strains as potential probiotics and subsequent confirmation of the in vivo effectiveness of Bacillus subtilis MA139 in pigs. *Antonie Van Leeuwenhoek* **2006,** *90* (2), 139-46.
23. (a) Aly, S. M.; Abdel-Galil Ahmed, Y.; Abdel-Aziz Ghareeb, A.; Mohamed, M. F., Studies on Bacillus subtilis and Lactobacillus acidophilus, as potential probiotics, on the immune response and resistance of Tilapia nilotica (Oreochromis niloticus) to challenge infections. *Fish Shellfish Immunol* **2008,**

25 (1-2), 128-36; (b) Balcazar, J. L.; Rojas-Luna, T., Inhibitory activity of probiotic Bacillus subtilis UTM 126 against vibrio species confers protection against vibriosis in juvenile shrimp (Litopenaeus vannamei). *Curr Microbiol* **2007,** *55* (5), 409-12; (c) Kumar, R.; Mukherjee, S. C.; Prasad, K. P.; Pal, A. K., Evaluation of Bacillus subtilis as a probiotic to Indian major carp Labeo rohita (Ham.). *Aquac Res* **2006,** *37* (12), 1215-1221.

CHAPTER 3

Sea Lions Develop True Vernix Caseosa Delivering Branched Fatty Acids and Squalene to the GI Tract*

Abstract

Vernix caseosa, the white waxy coating found on newborn human skin, is thought to be a uniquely human substance. Its signature characteristic is exceptional richness in saturated branched chain fatty acids (BCFA) and squalene. Vernix particles sloughed from the skin suspended in amniotic fluid are swallowed by the human fetus, depositing BCFA/squalene throughout the gastrointestinal (GI) tract [1], thereby establishing a unique microbial niche that influences development of nascent microbiota [2]. Here we show that late-term California sea lion (*Zalophus californianus*) fetuses have true vernix caseosa, delivering BCFA and squalene to the fetal GI tract thereby recapitulating the human fetal GI tract microbial niche. These are the first data demonstrating the production of true vernix caseosa in a species other than *Homo sapiens*. Its presence in a marine mammal supports an aquatic habituation period in the evolution of modern humans.

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Introduction

Vernix caseosa ("cheesy varnish") is the white, fat laden material found on the skin of human newborns, long thought to be unique to humans [3]. Vernix is synthesized by the fetal skin sebaceous glands, and is approximately half lipid on a dry matter basis, including shed fetal skin cells [4]. The fatty acyl chains of the lipid component is unique among human substances, containing about 30% saturated monomethyl branched chain fatty acids (BCFA) with branching near the terminal end of the acyl chains[1]. Vernix BCFA characteristically consist of a broad distribution of acyl chain lengths from about C_{11} to C_{26}, similar to other BCFA distributions originating in the skin such as lanolin (sheep wool fat). Milks are other major mammalian substances with significant concentrations of monomethyl BCFA, having a much narrower range of chain lengths. Bovine [5], goat [6], or yak [7] milk BCFA have chain lengths from about C_{14} to C_{18} biosynthesized by rumen bacteria, and present at about 2-6% of total fatty acids. Human breast milk fat contains 1-2% [8]. The distribution of BCFA chain lengths serves as a marker of their origin: BCFA with chain length distributions within range of C_{14}-C_{18} originate in milks, while distributions outside this range originate in skin. In about the last third of gestation, vernix particles slough off the skin where it causes amniotic fluid to become increasingly turbid, reaching a maximum particle density at about week 37 of the normal 40 week human gestation. Squalene, another component of human surface lipids is rare in other species, having been reported in the surface lipids of only 4 mammals related to aquatic systems out of more than

60 species analyzed to date [9]. Squalene comprises about 12% surface lipids in human adults [10] and is also a component of human vernix caseosa, at about 9% [11].

The biological role of vernix is not well understood; most hypotheses are related to skin function. Vernix is thought to be a barrier to water loss, assists in neonatal temperature regulation, and in innate immunity including antibacterial activity [12]. A complementary hypothesis is that vernix serves a nutritional role as a non-fermentable prebiotic, and alters the physiology of constituent microorganisms. Through the last trimester, human fetuses actively swallow hundreds of milliliters of amniotic fluid daily, and with it vernix particles. In this respect vernix caseosa can be considered the first solid meal of humans. Vernix BCFA are found in meconium, the first fecal excretion of newborns, with chain lengths corresponding to higher molecular masses [1]. BCFA are therefore at high concentration throughout the newborn GI tract as the first organisms are arriving to colonize the gut. BCFA are major constituents of the membranes of many microorganisms, reaching 95% of fatty acyl chains and are particularly rich in most species in the *bacilli* genus. In membranes, BCFA serve similar biophysical functions as *cis* unsaturated double bonds, lowering phase transition temperatures while avoiding vulnerability to oxygen attack of double bonds and their active allylic sites [13]. In a newborn rat pup model of necrotizing enterocolitis (NEC), BCFA treatment shifted the distribution of the nascent microbiota toward organisms known to use BCFA in their membranes while reducing the incidence of NEC by >50% [2]. BCFA are also known *in vitro* to reduce motility, and presumably virulence, of the pathogen *Pseudomonas aeruginosa* [14]. As a major component of human vernix caseosa, squalene was found to have some similar biological activities such as skin hydration, antitumor effects and as an emollient and antioxidant [15].

In May of 2013, an algal bloom caused a domoic acid event off the coast of Southern California resulting in late-term abortions and maternal death of wild California sea lions. We examined fetuses from these animals at a stranded animal facility in San Diego, California. **Figure 3.1** is a late-term California sea lion (*Zalophus californianus*) fetus collected from a deceased pregnant female with a human newborn for comparison. The sea lion back shows a patchy white film similar to the appearance of vernix caseosa on human newborns. The whiskers in particular display small clumps of white material notable at the base. The clumping is similar to that on eyebrows and lanugo, fine fetal body hair, of humans.

Figure 3. 1   Vernix caseosa visible on a late-term California sea lion fetus and a human newborn.

(A) The sea lion has white debris evident on the whiskers, eyebrows, head, and neck, and (B) along the back. (C) A human newborn seconds old, with vernix caseosa on most of the skin.

We hypothesized that this material is the sea lion equivalent of human vernix caseosa by measuring fatty acyl distribution to establish BCFA distribution and abundance as well as squalene concentrations.

Methods

Ethical approval

This work was authorized under the US Marine Mammal Protection Act; samples were collected under National Marine Fisheries Scientific Research Permit No. 932-1489-00.

Sample collection

Vernix, amniotic fluid, gastric content, meconium, serum of late to full-term were collected during necropsies from six sea lion pregnant animal/fetal pairs. Additional meconium and gastric content were obtained from other pairs in the same manner for squalene analyses. All tissues and fluids were stored in dry ice or at -80 C until analysis.

Fatty acid analysis

Total lipids were extracted from meconium, amniotic fluid, and gastric contents according to a routine method as described in detail elsewhere [5].

Results

The chemical composition of characteristic BCFA and squalene confirmed the observational vernix caseosa for California sea lions. Samples were collected at necropsy from six sea lion fetuses, two males and four females, of weights 2.75 to 5.50 kg and lengths 46 to 67 cm length. The percentage of BCFA found in total lipids of amniotic fluid and meconium increased sigmoidally with fetal weights (Figs. 2A and 2B). From our regression model, it was found that BCFA were staying at about 1% in both amniotic fluid and meconium at the very early stage of fetus development and BCFA concentration throughout the uterus-gut environment increased sharply at the critical weight of 2.5 kg. The BCFA accumulation reached a peak concentration when the fetus developed into late term, about 4-5 kg in total weight and then BCFA concentration plateaued and maintained at high concentration near parturition. In the heaviest fetus (late term), amniotic lipid BCFA was 17.9%, while meconium lipid was 11.3%. The pattern is similar to that in human fetuses, which begin to release BCFA-containing vernix particles into the amniotic fluid until the second half of gestation; human meconium BCFA is 17.5%, very similar to that of the heaviest fetal sea lion [1]. On the other hand, squalene content in the same sample types increased linearly with gestational weight gain (Figs. 2C and 2D). From the linear regression model of both amniotic fluid and meconium, we extrapolate that the production of squalene starts at a weight gain of 2.3 kg for California sea lion fetuses.

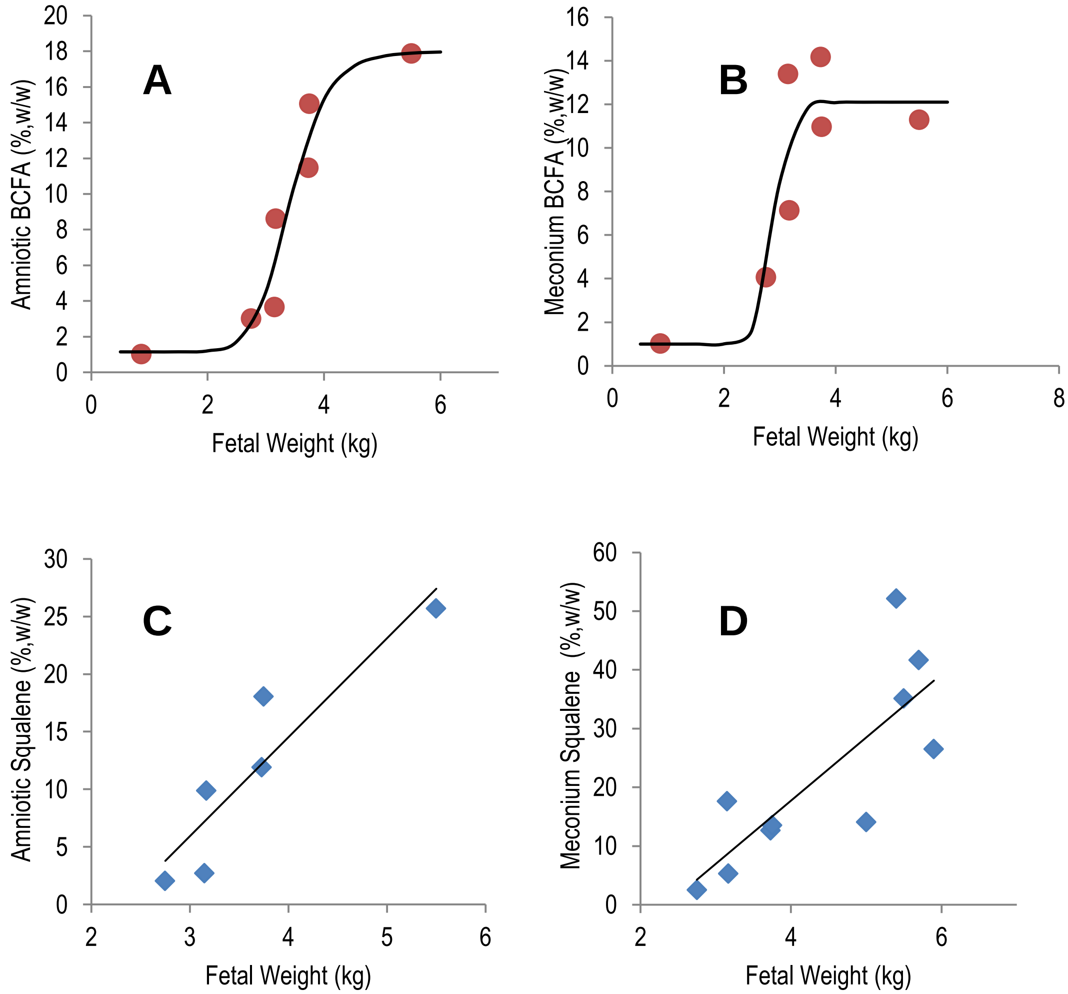

Figure 3. 2 BCFA and squalene in amniotic fluid and meconium increase with gestational weight gain.

(A)The sigmoidal shapes are consistent with development of vernix in the last half of gestation, as in humans. Meconium only was available from 0.86 kg early-gestation sea lion fetus and is plotted for amniotic fluid modeling. B = 18.0 + (1.15 – 18.0)/(1 + (w/3.42)^10.6); r^2 = 0.93; p=0.04. B is total BCFA, and w is fetal weight. (B) Meconium BCFA. B = 12.1 + (0.997 - 12.1)/(1 + (w/2.89)^19.1); r^2=0.81, p=0.18. (C) Squalene in amniotic fluid shows a linear growth trend. $S=8.59w-19.8$; $r^2=0.84$; $p<0.001$. (D) Squalene in meconium. $S=10.8w-25.4$; $r^2=0.64$; $p<0.001$.

The distribution of BCFA in vernix, amniotic fluid, gastric contents, and meconium averaged (n=6) sea lion fetuses shows a distribution of chain lengths from C_{11} to C_{24}, and consisting of *iso* and *anteiso* isomers (**Figure 3.3A**), indicative of origin on the skin and not milk. Similar to human vernix and meconium (**Figure S3.1A**), sea lion vernix and meconium BCFA are rich in *iso* BCFA, showing a dominant pattern suggesting two carbon elongation in the sequence *iso*-16:0 → *iso*-18:0 → *iso*-20:0 → *iso*-22:0 → *iso*-24:0. *iso*-20:0 was at highest concentration in all pools, ranging from 2.3% in stomach contents to 4.7% in meconium. *iso*-20:0 and *iso*-22:0 comprised 5.1% and 7.2% of sea lion vernix and meconium, respectively, similar to human vernix and meconium of 3.1% and 6.5% *iso*-20:0 and *iso*-22:0. In contrast, cow's milk, another major BCFA containing substance, has BCFA from C_{14}-C_{18} [16] (**Figure S3.1B**) and milks of Atlantic grey seals [17], Antarctic fur seals [17], stellar sea lions [18], and New Zealand sea lions [19] have BCFA from C_{15}-C_{18}. Fetal sea lion serum BCFA were also detected, however in contrast to the other substances, the maximal BCFA was *iso*-17:0 at 0.35%, again consistent with low BCFA detected in human plasma [20]. Levels of total BCFA for the three heaviest fetuses range from 4.7 to 14.8% for gastric contents and amniotic fluid, respectively, while serum total BCFA was 1.3% (**Figure 3.3, inset**).

We consider squalene as a percentage of total fatty acids as a convenient measure. Squalene reaches a peak of 22%, w/w in meconium, while it is never more than at trace levels in serum. Statistical analysis shows that serum squalene is significantly lower than meconium, vernix and amniotic fluid. Meconium squalene is significantly higher than squalene content in stomach fluid. Mean squalene values for amniotic fluid and stomach content are 12% and 8%, respectively. Of the four heaviest fetuses, meconium squalene is as high as 40%. Of the only pup that approached term weight of 6 kg, amniotic fluid is about 25%. Vernix squalene is 15% (**Figure 3.3B**), similar to its level in human vernix (~9%) [11].

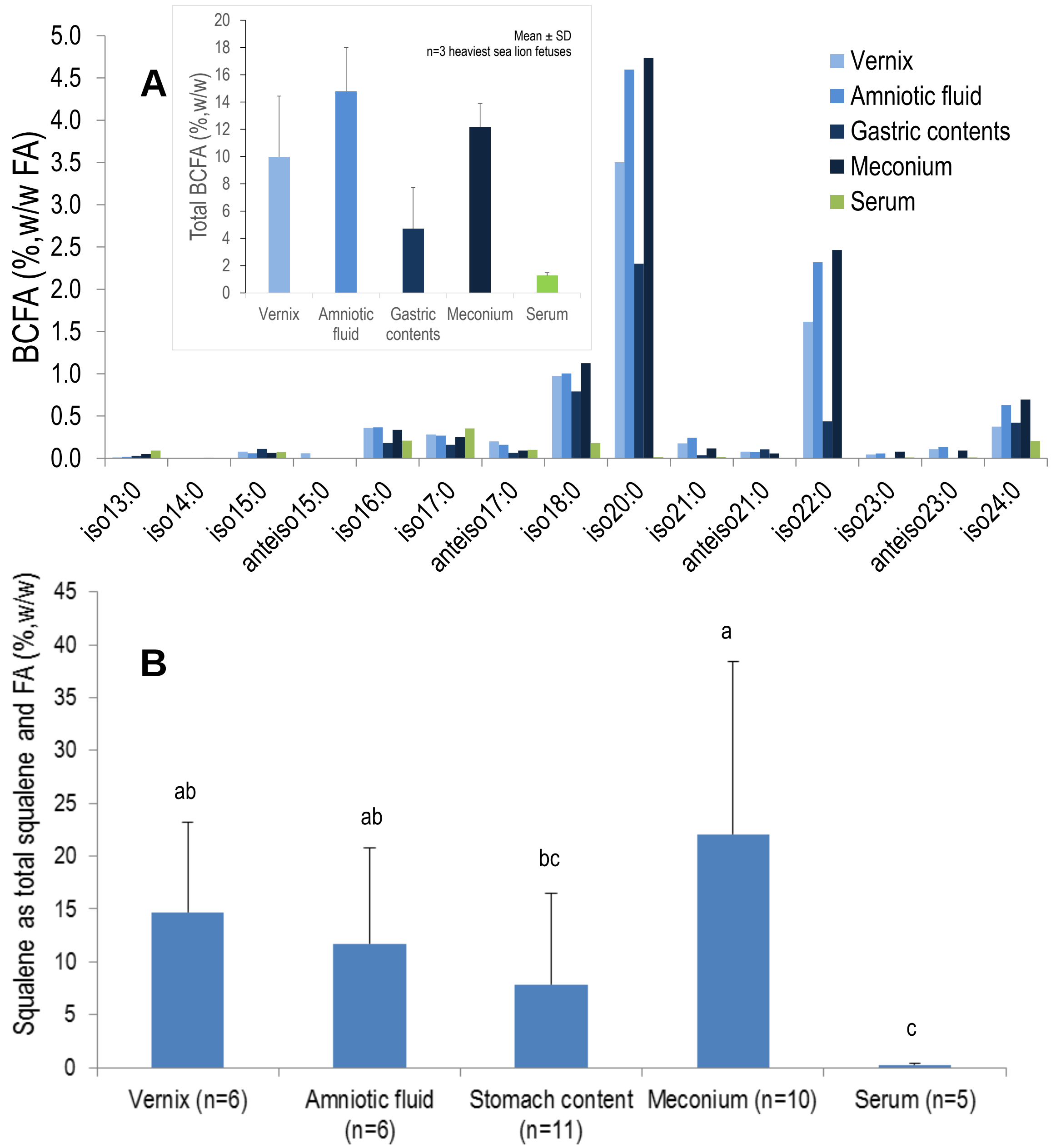

Figure 3. 3 California sea lion fetal BCFA/squalene in vernix, amniotic fluid, gastric content, meconium, serum (n=6).

(A) *iso*-BCFA C_{18-24} dominate the distribution, similar to human BCFA, and unlike milkfat distributions C_{14-18} (**Figure S3.2**). Serum BCFA distribution is dissimilar, $C_{16-18,24}$, showing BCFA are only selectively transported to the circulation. Technical CV averages 3%. Inset: Summed BCFA in each substance for the three heaviest fetuses average about 10% except for serum. (B) Squalene is high in all substances except serum. Different letters signify a significant difference.

Discussion

This study is the first to find that vernix caseosa is not unique to human beings and at least one species of marine mammal, California sea lion shares this physiological and evolutional characteristic with us. The existence of skin originated long chain BCFA and squalene, in various samplings including amniotic fluid, stomach content, meconium and vernix itself throughout the sea lion gut, consolidates the observation of physically resembled vernix on the surface of sea lion pups.

It is surprising to tell that some species that are thought to be closely related to human beings lack vernix caseosa, for instance, chimpanzee and baboon. The production of BCFA may parallel the loss of hair in these mammals, as we already see in California sea lion and human beings. However, BCFA can serve another probably even more important function for us and California sea lion considering its prevalence in the GI tract. This speculation comes from a study which demonstrates beneficial effects of BCFA to neonatal rats in terms of reducing induced necrotizing enterocolitis [2]. However, as far as we know rat doesn't produce vernix caseosa. More research either with human or California sea lion is needed to demonstrate if vernix caseosa really improves the gut health of its host, in a natural way.

Squalene has long been known to be a major component of human surface lipids. Of at least 60 mammals reported to date, squalene has been found in the sebum of only four other mammals: beavers and otters – two aquatic mammals, the kinkajou – a nocturnal arboreal rainforest dweller, and the mole.

Early authors investigating surface lipid classes have observed that the squalene is only found in animals that inhabit a “damp environment” suggesting a function for squalene in skin lipids of mammals whose surface is often wet [9a]. California sea lions are now the sixth species.

Sir David Attenborough raises the issue of vernix as one of the many human traits parallel to aquatic adaptation of marine mammals [21]. The role of aquatic resources in human evolution is controversial, inspired recently by the nutritional considerations for supporting human brain growth [22]. The unique observation of BCFA/squalene in the GI tract in newborns suggests an advantage to shifting properties of the nascent microbiota in a way shared by marine mammals and humans, and possibly a metabolic parallel in enterocytes or other intestinal function.

REFERENCES

1. Ran-Ressler, R. R.; Devapatla, S.; Lawrence, P.; Brenna, J. T., Branched chain fatty acids are constituents of the normal healthy newborn gastrointestinal tract. *Pediatric research* **2008,** *64* (6), 605-9.

2. Ran-Ressler, R. R.; Khailova, L.; Arganbright, K. M.; Adkins-Rieck, C. K.; Jouni, Z. E.; Koren, O.; Ley, R. E.; Brenna, J. T.; Dvorak, B., Branched chain fatty acids reduce the incidence of necrotizing enterocolitis and alter gastrointestinal microbial ecology in a neonatal rat model. *PloS one* **2011,** *6* (12), e29032.

3. Hoath, S. B.; Pickens, W. L., The Biology of Vernix. In *Neonatal Skin: Structure and Function*, 2 ed.; Hoath, S. B.; Maibach, H. I., Eds. Marcel Dekker: New York, 2003.

4. Pickens, W. L.; Warner, R. R.; Boissy, Y. L.; Boissy, R. E.; Hoath, S. B., Characterization of vernix caseosa: water content, morphology, and elemental analysis. *J Invest Dermatol* **2000,** *115* (5), 875-81.

5. Ran-Ressler, R. R.; Bae, S.; Lawrence, P.; Wang, D. H.; Brenna, J. T., Branched-chain fatty acid content of foods and estimated intake in the USA. *The British journal of nutrition* **2014,** *112* (4), 565-72.

6. DePooter, H.; Decloedt, M.; Schamp, N., Composition and variability of the branched-chain fatty acid fraction in the milk of goats and cows. *Lipids* **1981,** *16* (5), 286-92.

7. Or-Rashid, M. M.; Odongo, N. E.; Subedi, B.; Karki, P.; McBride, B. W., Fatty acid composition of yak (Bos grunniens) cheese including conjugated linoleic acid and trans-18:1 fatty acids. *J Agric Food Chem* **2008,** *56* (5), 1654-60.

8. (a) Gibson, R. A.; Kneebone, G. M., Fatty acid composition of human colostrum and mature breast milk. *Am J Clin Nutr* **1981,** *34* (2), 252-7; (b) Egge, H.; Murawski, U.; Ryhage, R.; Gyorgy, P.; Chatranon, W.; Zilliken, F., Minor constituents of human milk. IV. Analysis of the branched chain fatty acids. *Chem Phys Lipids* **1972,** *8* (1), 42-55.

9. (a) Downing, D. T.; Stewart, M. E., Skin surface lipids of the mole Scalopus aquaticus. *Comp Biochem Physiol B* **1987,** *86* (4), 667-70; (b) Lindholm, J. S.; Mccormick, J. M.; Colton, S. W.; Downing, D. T., Variation of Skin Surface Lipid-Composition among Mammals. *Comp Biochem Phys B* **1981,** *69* (1), 75-78; (c) Nicolaides, N.; Fu, H. C.; Rice, G. R., The skin surface lipids of man compared with those of eighteen species of animals. *J Invest Dermatol* **1968,** *51* (2), 83-9.

10. (a) Cotterill, J. A.; Cunliffe, W. J.; Williamson, B.; Bulusu, L., Age and sex variation in skin surface lipid composition and sebum excretion rate. *Br J Dermatol* **1972,** *87* (4), 333-40; (b) Ramasastry, P.; Downing, D. T.; Pochi, P. E.; Strauss, J. S., Chemical composition of human skin surface lipids from birth to puberty. *J Invest Dermatol* **1970,** *54* (2), 139-44.

11. Kaerkkaeinen, J.; Nikkari, T.; Ruponen, S.; Haahti, E., Lipids of Vernix Caseosa. *J Invest Dermatol* **1965,** *44*, 333-8.

12. Visscher, M. O.; Narendran, V.; Pickens, W. L.; LaRuffa, A. A.; Meinzen-Derr, J.; Allen, K.; Hoath, S. B., Vernix caseosa in neonatal adaptation. *J Perinatol* **2005,** *25* (7), 440-6.

13. Kaneda, T., Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. *Microbiological reviews* **1991,** *55* (2), 288-302.

14. Inoue, T.; Shingaki, R.; Fukui, K., Inhibition of swarming motility of Pseudomonas aeruginosa by branched-chain fatty acids. *FEMS Microbiol Lett* **2008,** *281* (1), 81-6.

15. Huang, Z. R.; Lin, Y. K.; Fang, J. Y., Biological and pharmacological activities of squalene and related compounds: potential uses in cosmetic dermatology. *Molecules* **2009,** *14* (1), 540-54.

16. Ran-Ressler, R. R.; Sim, D.; O'Donnell-Megaro, A. M.; Bauman, D. E.; Barbano, D. M.; Brenna, J. T., Branched chain fatty acid content of United States retail cow's milk and implications for dietary intake. *Lipids* **2011,** *46* (7), 569-76.

17. Ackman, R. G.; Burgher, R. D., Component Fatty Acids of the Milk of the Grey (Atlantic) Seal. *Can J Biochem Physiol* **1963,** *41*, 2501-5.

18. Miller, C. N. Milk Fatty Acid Composition of Perinatal and Foraging Steller Sea Lions: Examination from Pup Stomachs. thesis, University of Alaska, Fairbanks, AK, 2014.

19. Riet-Sapriza, F. G. Milk Composition of the New Zealand Sea Lion and Factors that Influence It. thesis, Massey University, Palmerston North, 2007.

20. Mika, A.; Stepnowski, P.; Kaska, L.; Proczko, M.; Wisniewski, P.; Sledzinski, M.; Sledzinski, T., A comprehensive study of serum odd- and branched-chain fatty acids in patients with excess weight. *Obesity (Silver Spring)* **2016,** *24* (8), 1669-76.

21. (a) Attenborough, D.; Collins, R., Scars of Evolution. *BBC Radio 4* **2004**; (b) Attenborough, D.; Collins, R., The Waterside Ape. *BBC Radio 4* **2016**.

22. Cunnane, S. C.; Crawford, M. A., Energetic and nutritional constraints on infant brain development: implications for brain expansion during human evolution. *J Hum Evol* **2014,** *77*, 88-98.

CHAPTER 4

Overview of Food BCFA and Indications

It may be surprising to some people but BCFA are actually closely related to food science and human nutrition. People of all ages and different ethnicity consume significant amount of BCFA. Infants swallow BCFA loaded vernix caseosa during the last stage of their gestation. After they wean and enter adolescence and adulthood, western population frequently consume dairy products and beef which contain about 2% BCFA of total fatty acids[1]; Asian population consume some fermented Asian foods, which could be a major source of food BCFA, a typical example would be nātto or shrimp paste. Our study found an average of 1% BCFA in 27 freshwater fish species, indicating the couple of papers that reporting high fish BCFA concentration may have issues with BCFA peaks identification or could be due to samplings of uncommon/nonfood fishes. If considering fat content and serving size, fish contribute only a small amount of BCFA to our diet compared with milk, and nātto is so far the only non-ruminant food that contains comparable amount of BCFA per serving. (**Figure 4.1**)

Table 4. 1 A standard portion of fish contains low BCFA compared to a serving of milk and nātto.

Freshwater finfish around NY	**BCFA (mg fatty acid/70 g fillet)**
Rock bass	12

Lake trout	17
Chain pickerel	5
Bowfin	8
Walleye	9
Fantail darter	3
Slimy sculpin	3
Black crappie	7
Common shiner	6
Rainbow smelt	11
Longnose dace	5
One cup of whole milk (244g=8 oz)	**158**[2]
Nātto (90g)	**117**

It is very interesting that vernix caesosa, the first meal of both human and California sea lion, contains 30%[3] and 10% of BCFA, a level much higher than any common food. BCFA and squalene increase with gestation time, similar to human infants. Long chain BCFA and squalene serve as biomarkers for true vernix caseosa so we established that human and sea lions shared the same trait of swallowing vernix into the GI tract. When it comes to BCFA, more attention is paid on the *iso* or *anteiso* difference and less on the chain length distribution of food BCFA and skin derived BCFA. Cow's milk[2], goat's milk[4], yak's milk[5], camel's milk, moose's milk[6], other dairy products, beef[1], camel meat[7], fish and nātto mainly contain BCFA from C14-C18. Skin surface BCFA from various species including human beings[3], California sea lions, rat[8], mouse, hamster[9], monkey[10], horse[11] and dog[12] are high proportionally in *iso*-20:0 and longer chain BCFA, and in most cases there long chain BCFA are most concentrated in wax ester or sterol ester fraction. Therefore, it is very likely that bacterial BCFA or food BCFA cannot satisfy the biological functions of skin and apparently a unique elongation pathway exists for sebaceous glands. Elongases 1-7 were identified in human tissues and were suggested to have different and harmonious functions in extending the chain length of fatty acids. Studies are undertaken in our lab to determine the elongation activity of the above mentioned elongases in human intestinal cell culture. Further research can be done to determine which elongase is responsible for making long chain BCFA in human skin and skins of other mammals. Establishing their levels of conservation for these enzymes and genes could shed more light on the evolutionary aspects of skins between various animal models.

Future studies should determine the health effects of BCFA, e.g. its functions on the skin and its interactions with microbiota and enterocytes. Skin care and cosmetic industry is growing rapidly in recently years with plant or animal extracts blooming in the market. While being extremely profitable, many of these new products lack enough evidence that they could promote skin well-being. Instead of resorting to more and more exotic oil extracts for the growth of the industry, researcher and product developer can go back to the human skin for more ideas, e.g. BCFA. Branched chain fatty acids have the advantage of the least vulnerability of oxidation and enhance membrane fluidity, just as the cis double bonds do. Researchers can design studies to investigate if BCFA can minimize damage to the skins by many stresses, such as UV-light, cleaning chemicals, heat or frostbite. The interactions of BCFA and gut microbiota are bound to become a new area of scientific research, considering the increasing understanding of the importance of microbiota and the infusion of BCFA to the gut when the first microorganisms colonize the infants. Bacteria species such as those in the bacillus genus and many lactic acid bacteria biosynthesize and utilize huge amounts of BCFA[13]. While the microbiota can be individually unique and modified through one's life span by diet change, life style change, antibiotic, disease, etc., the first few hours after born when large quantities of vernix BCFA persist in the GI tract is likely to be the most critical for the establishment of the composition of the infant's microbiota. This first arriving colony is also most influential to one's later health condition, immunity, nutrient absorption and even personality. It can be speculated that high concentrations of BCFA may be essential to the selection of this first colony and may be a feature of human-microbial symbiotic evolution.

More research on BCFA's functionality would not only have significance in human health promotion and understanding human evolution, but also in new food products development. The methyl branch of a branched chain fatty acid prevents chain close association. As a result, intermolecular forces and melting points are reduced, a phenomenon similar to what cis double bonds do. However, there is no vulnerability to oxidation or rancidity with BCFA. *Anteiso* is more effective than *iso* at reducing melting point and the effect is greater on diacylphosphatidylcholine than non-esterified fatty acid. A diacylphosphatidylcholine with normal 17:0 as the acyl groups has a transition temperature of 49 °C and substituting them with *iso*-17:0 would reduce the transition temperature to 27 °C. If *anteiso*-17:0 is used as the acyl component, it would further decrease to 8 °C, rendering the molecule liquid form in most ambient temperature except for some harsh winter temperature[13]. A recent paper simulated the effects of methyl branching on a dipalmitoylphosphatidylcholine and found that branching reduces lipid condensation, decreases the bilayer thickness and lowers chain ordering. It also results in the formation of kinks due to the branching and thus increases the fluidity of the lipid bilayers. In addition, they also found that polymethylated chains, if the gap between methyl groups is as wide as 2-3 carbons, the effects of the methyl branches are additive[14]. All these findings pave the way to develop new food ingredients such as a new version of frying oil. Although lack of preliminary data of any kind, we can expect some great advantage of BCFA frying oil over traditional frying oil, which is basically soybean oil.

Soybean oil contains high amount of polyunsaturated and monounsaturated fatty acids, which keep it liquid at room temperature. However, polyunsaturated fatty acids are very unstable and can be easily oxidized especially at frying temperature. In contract, if those polyunsaturated fatty acids such as linoleic acid can be replaced with one or more BCFA such as *anteiso*-17:0, we can expect a longer shelf life and least off flavors, while the oil can stay in liquid form due to the kinks in BCFA chain. Some emerging frying oil as high oleic frying oil has come to the market, and it surely improves its stability over traditional frying oil. However, saturated BCFA oil could offer even better shelf life due to its least vulnerability to oxygen attacks.

The major problem preventing researchers from creating the ideal frying oil from BCFA is its source. The only two known sources of BCFA are bacteria and skin of some species. Except vernix, skin is usually not very abundant in BCFA. BCFA often concentrate in wax ester or sterol ester fractions and it may not be worth separating out for mass production. Bacteria BCFA may be more promising for industrial level production. Kaneda has summarized hundreds of BCFA containing bacteria that researchers can refer to[13].

All in all, our study demonstrated that BCFA containing foods are not limited to dairy and beef products. BCFA functions to GI tract and skin would be an important to dermatology, nutrition, neonatology and pediatrics, gastroenterology, as well as mammalian evolution.

REFERENCE

1. Ran-Ressler, R. R.; Bae, S.; Lawrence, P.; Wang, D. H.; Brenna, J. T., Branched-chain fatty acid content of foods and estimated intake in the USA. *The British journal of nutrition* **2014,** *112* (4), 565-72.

2. Ran-Ressler, R. R.; Sim, D.; O'Donnell-Megaro, A. M.; Bauman, D. E.; Barbano, D. M.; Brenna, J. T., Branched chain fatty acid content of United States retail cow's milk and implications for dietary intake. *Lipids* **2011,** *46* (7), 569-76.

3. Ran-Ressler, R. R.; Devapatla, S.; Lawrence, P.; Brenna, J. T., Branched chain fatty acids are constituents of the normal healthy newborn gastrointestinal tract. *Pediatric research* **2008,** *64* (6), 605-9.

4. (a) DePooter, H.; Decloedt, M.; Schamp, N., Composition and variability of the branched-chain fatty acid fraction in the milk of goats and cows. *Lipids* **1981,** *16* (5), 286-92; (b) Klir, Z.; Castro-Montoya, J. M.; Novoselec, J.; Molkentin, J.; Domacinovic, M.; Mioc, B.; Dickhoefer, U.; Antunovic, Z., Influence of pumpkin seed cake and extruded linseed on milk production and milk fatty acid profile in Alpine goats. *Animal* **2017**, 1-7.

5. Or-Rashid, M. M.; Odongo, N. E.; Subedi, B.; Karki, P.; McBride, B. W., Fatty acid composition of yak (Bos grunniens) cheese including conjugated linoleic acid and trans-18:1 fatty acids. *J Agric Food Chem* **2008,** *56* (5), 1654-60.

6. Dreiucker, J.; Vetter, W., Fatty acids patterns in camel, moose, cow and human milk as determined with GC/MS after silver ion solid phase extraction. *Food chemistry* **2011,** *126* (2), 762-771.

7. Rawdah, T. N.; Zamil El-Faer, M.; Koreish, S. A., Fatty acid composition of the meat and fat of the one-humped camel (camelus dromedarius). *Meat Sci* **1994,** *37* (1), 149-55.

8. Oku, H.; Oohama, E.; Yagi, N.; Urahashi, A.; Nagata, J.; Shinjyo, C.; Chinen, I., Age-related changes of the branched-chain fatty acid concentration in rat skin surface lipid. *Comp Biochem Physiol B* **1992,** *102* (2), 357-62.

9. Oku, H.; Nakanishi, T.; Kumamoto, K.; Chinen, I., Distribution of branched-chain fatty acid in the skin surface lipid of laboratory animals. *Comp Biochem Physiol B* **1990,** *96* (3), 475-82.

10. Nishimaki-Mogami, T.; Minegishi, K.; Takahashi, A.; Kawasaki, Y.; Kurokawa, Y.; Uchiyama, M., Characterization of skin-surface lipids from the monkey (Macaca fascicularis). *Lipids* **1988,** *23* (9), 869-77.

11. Downing, D. T.; Colton, S. W. t., Skin surface lipids of the horse. *Lipids* **1980,** *15* (5), 323-7.

12. Sharaf, D. M.; Clark, S. J.; Downing, D. T., Skin surface lipids of the dog. *Lipids* **1977,** *12* (10), 786.

13. Kaneda, T., Iso- and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. *Microbiological reviews* **1991,** *55* (2), 288-302.

14. Poger, D.; Caron, B.; Mark, A. E., Effect of methyl-branched fatty acids on the structure of lipid bilayers. *J Phys Chem B* **2014,** *118* (48), 13838-48.

APPENDIX

Table S1.1. Locations and dates of capture, length and number of each of the 27 fish species from the northeastern United States

Common name (Scientific name)	location	Date of capture	Length (cm)	Sample size	Notes*
Rainbow smelt (*O. mordax*)	Adiron	5/19/2015	6,6,6	3	Fished commercially, feed on invertebrates and zooplankton
Pumpkinseed (*L. gibbosus*)	Oneida Lake	Jun-Jul 2015	6,7,7,17,20,20	6	Feed on aquatic invertebrates
White sucker (*C. commersonii*)	Adiron/Whitney	May-Jul 2015	7,7,7,16,35,43,44	7	Feed on benthic invertebrates
Lake trout (*S. namaycush*)	Cayuga Lake	7/31/2015	33,35,37	3	Piscivorous
Freshwater drum (*A. grunniens*)	Oneida Lake	7/28/2015	33,41,44	3	Feed on fish, crayfish and mollusks
Alewife (*A. pseudoharengus*)	Cayuga Lake	7/31/2015	13,14,15	3	Feed on zooplankton and aquatic insects
Common shiner (*L. cornutus*)	Adiron	5/19/2015	7,7,7	3	Stream fish, feed on insect larvae
White crappie (*P. annularis*)	Whitney	7/7/2015	22,23,23	3	Feed on aquatic insects and fish
Walleye (*S. vitreus*)	Whitney	7/7/2015	22,29,42	3	Fished commercially, piscivorous
Channel catfish (*I. punctatus*)	Oneida Lake	8/4/2015	28,33,33	3	Popular food fish, feed on fish and insects
Greater redhorse (*M. valenciennesi*)	Oneida Lake	7/21/2015	42,48,51	3	Feed on benthic invertebrates
Black crappie (*P. nigromaculatus*)	Bald Eagle Creek	5/14/2015	19,20,21	3	Feed on aquatic insects and fish
Smallmouth bass (*M. dolomieu*)	Adiron/Whitney	May-Jul 2015	6,6,6,24,43	5	Feed on fish and crayfish
Golden shiner (*N. crysoleucas*)	Whitney	7/7/2015	18,23	2	Feed on zooplankton and algae
Slimy sculpin (*C. cognatus*)	Cascadilla Creek	5/9/2015	8,12	2	Feed on benthic invertebrates
Brown bullhead (*A. nebulosus*)	Whitney	7/7/2015	34,34,36	3	Fished commercially, feed on invertebrates

Redbreast sunfish (*L. auritus*)	W. Susquehanna	7/9/2015	14,15,16	3	Feed on zooplankton and aquatic insects
Blacknose dace (*R. atratulus*)	Cascadilla Creek	5/9/2015	9,10,11	3	Stream fish, feed on aquatic insects
Rock bass (*A. rupestris*)	Whitney/Tusca	Apr-Jul 2015	13,14,15,16,17,17, 18,22	8	Feed on fish and aquatic insects
Longnose dace (*R. cataractae*)	Cascadilla Creek	5/9/2015	10,10,11,11,11	5	Stream fish, feed on aquatic insects
Fantail darter (*E. flabellare*)	Cascadilla Creek	5/9/2015	6,7,7,7	4	Stream fish, feed on aquatic insects
Bowfin (*A. calva*)	Oneida Lake	9/18/2015	50,51,58	3	Ancient fish, feed on fish and crayfish
Chain pickerel (*E. niger*)	Oneida Lake	7/28/2015	47,47,55	3	Feed on fish and crayfish
White perch (*M. Americana*)	Oneida Lake	6/9/2015	19,20,20,20,21,21	6	Feed on aquatic insects and zooplankton
Burbot (*L. lota*)	Oneida Lake	9/15/2015	50,50,50	3	Piscivorous
Yellow perch (*P. flavescens*)	Whitney	7/7/2015	26,28,28	3	Fished commercially, Feed on aquatic insects, zooplankton and fish
Bluegill (*L. macrochirus*)	Whitney	7/7/2015	19,20,20,21,21	5	Feed on aquatic insects

Abbreviations for locations: Adirondack, NY (Adiron); Whitney Point Reservoir, NY (Whitney); Bald Eagle Creek, PA; Cascadilla Creek , Ithaca, NY; W. Susquehanna, PA; Tuscarora Creek, PA (Tusca).

Fish species are listed from highest to lowest BCFA content in fish muscle, and this order is followed in all tables.

* Diet information was drawn from Smith, C. L., 1985 [1]

Table S1.2. Fatty acid retention time (min) of a sample and combined standards from the same GC-FID run

	Common shiner	Standards
12:0	4.336	
i13:0	4.516	
ai13:0	4.576	
13:0	4.686	
i14:0	4.886	4.893
14:0	5.083	5.08
14:1n-5	5.243	5.313
i15:0	5.306	
ai15:0	5.386	5.433
15:0	5.536	
i16:0	5.813	5.84
16:0	6.23	6.11
16:1n-7	6.456	6.386
i17:0	6.456	
ai17:0	6.613	6.626
phytanic acid	6.726	
17:0	6.823	
16:2	6.926	
17:1n-8	7.09	
i18:0	7.17	7.223
18:0	7.64	7.55
18:1n-9	7.893	7.796
18:2n-6	8.323	8.25
18:2	8.436	
18:3n-6	8.566	8.546
18:3	8.743	
18:3n-3	8.84	8.803
20:0	9.04	9.053
18:4n-3	9.116	
20:1n-9	9.32	9.283
20:1n-7	9.386	
20:2n-6	9.733	9.716
20:3n-6	10.006	9.99
20:4n-6	10.226	10.17
20:3n-3		10.24
20:4n-3	10.53	
22:1n-9		10.65
20:5n-3	10.773	10.7
22:2n-9		11.173
23:0I.S.	11.393	
22:4n-6	11.74	11.603
24:1		11.9
22:5n-3	12.133	12.11
22:6n-3	12.27	12.21
24:5	13.36	
24:6	13.59	

Table S1.3a. Normal saturated FA of fish muscle (weight %).

Fatty acid %wt	12:0	13:0	14:0	15:0	16:0	17:0	18:0	20:0	22:0	Total straight SFA
Rainbow smelt	0.07	0.05	2.49	0.66	18.62	0.47	3.61	0.10		26.07
Pumpkinseed	0.03	0.03	0.93	0.49	23.43	0.89	7.58	0.34	0.05	33.76
White sucker	0.03	0.01	0.93	0.49	23.47	0.70	4.48	0.25	0.12	30.48
Lake trout	0.02		1.30	0.42	25.50	0.44	4.58	0.16	0.01	32.43
Freshwater drum			0.84	0.40	19.64	0.79	10.98	0.52		33.18
Alewife	0.05	0.01	1.65	0.58	32.36	0.92	8.18	0.35	0.05	44.15
Common shiner	0.11	0.02	1.12	0.36	22.08	0.56	5.30	0.12		29.66
White crappie			1.38	0.68	26.24	0.99	8.88	0.35		38.51
Walleye	0.04	0.06	1.07	0.58	26.32	0.90	7.70	0.07		36.73
Channel catfish			0.88	0.37	23.45	0.64	8.51	0.17		34.01
Greater redhorse	0.05		1.04	0.30	24.55	0.48	8.29	0.13		34.84
Black crappie	0.14	0.06	1.02	0.36	22.39	0.58	4.83	0.10	0.03	29.52
Smallmouth bass			0.87	0.44	19.66	0.63	6.51	0.23		28.33
Golden shiner			0.41	0.30	24.87	0.85	7.45	0.18		34.06
Slimy sculpin	0.17	0.01	0.92	0.28	19.39	0.81	5.52	0.01		27.11
Brown bullhead			0.65	0.57	24.40	0.75	8.13	0.17		34.67
Redbreast sunfish			0.95	0.28	17.04	0.55	6.13	0.14		25.09
Blacknose dace	0.05	0.01	1.47	0.21	17.60	0.36	5.51	0.25	0.20	25.67
Rock bass	0.05	0.01	1.13	0.23	20.54	0.34	4.71	0.20		27.20
Longnose dace	0.02	0.05	0.68	0.29	20.78	0.57	5.35	0.14		27.88

Fantail darter	0.08	0.01	1.19	0.24	19.25	0.27	4.52	0.25		25.80
Bowfin			0.32	0.23	21.15	0.42	5.60	0.02		27.73
Chain pickerel			0.42	0.22	17.71	0.30	4.92	0.02		23.61
White perch	0.04	0.01	1.15	0.24	19.33	0.31	4.44	0.13		25.65
Burbot			0.64	0.19	19.03	0.17	5.88	0.05		25.97
Yellow perch			1.35	0.52	27.33	0.63	6.05	0.36		36.24
Bluegill			0.73	0.45	20.29	0.73	5.72	0.30		28.22
Mean CV (%)	66	72	21	18	9	17	11	64	140	7

Table S1.3b. Monounsaturated FA of fish muscle (weight %).

Fatty acid %wt	14:1n-5	16:1n-7 [a]	17:1n-8	18:1n-9 [b]	20:1n-9	20:1n-7	22:1n-9	24:1n-9	Total MUFA
Rainbow smelt		3.72	0.27	6.07	0.21	0.56		0.64	11.48
Pumpkinseed	0.09	5.34	0.37	10.72	0.82	0.18		0.15	17.66
White sucker	0.04	7.10	0.56	11.98	0.56	0.15		0.04	20.43
Lake trout		4.23	0.31	11.43	0.22	0.12		0.24	16.55
Freshwater drum		3.65		11.83	1.17			0.24	16.90
Alewife		2.81	0.21	9.60	0.94	0.12		0.24	13.92
Common shiner	0.08	6.19	0.52	14.21	0.36	0.07			21.43
White crappie		3.45		8.60	0.41			0.75	13.22
Walleye		4.28		9.88	0.16	0.02		0.41	14.74
Channel catfish		3.77	0.26	16.75	0.47	0.15		0.14	21.54
Greater redhorse	0.05	4.54	0.30	9.30	0.44	0.07		0.14	14.85
Black crappie	0.05	3.43	0.32	10.22	0.67	0.08		0.33	15.08
Smallmouth bass		3.81	0.57	10.52	0.58	0.26	0.01	0.69	16.45
Golden shiner		3.24		10.03	0.08	0.02		0.08	13.44
Slimy sculpin	0.09	6.02	0.59	10.96	0.48	0.11		0.01	18.25
Brown bullhead		3.36		10.60	0.13	0.08		0.61	14.78
Redbreast sunfish		4.48		14.57	0.28	0.01		0.22	19.55
Blacknose dace	0.07	6.55	0.46	11.89	0.37	0.28		0.06	19.68
Rock bass		4.50	0.11	9.68	0.32	0.07		0.13	14.81
Longnose dace	0.06	7.48		9.79	0.59	0.23	0.74	0.25	19.14

Fantail darter	0.11	7.38	0.48	9.02	0.38	0.10		0.01	17.48
Bowfin		4.09		18.34	0.06	0.04		0.44	22.97
Chain pickerel		2.24		9.90	0.06	0.02		0.40	12.63
White perch	0.02	3.94	0.66	8.51	0.68	0.02		0.17	14.01
Burbot		2.88		13.45	0.23	0.02		1.80	18.38
Yellow perch		5.29	0.65	7.60	0.16	0.02	0.06	0.58	14.37
Bluegill		3.15		11.58	0.21	0.04		0.54	15.51
Mean CV (%)	49	15	45	11	40	84	39	56	10

a. Smaller amount of 16:1n-9 and 16:1n-5 coelute with 16:1n-7

b. Smaller amount of 18:1n-7 coelute with 18:1n-9

Table S1.3c. Polyunsaturated FA of fish muscle (weight %).

Fatty acid %wt	16:2*	16:3*	18:2n-6	18:2*	18:3n-6	18:3*	18:3n-3	18:4n-3	20:2n-9	20:2n-6	20:3n-9	20:3n-6
Rainbow smelt			3.76		0.15		2.94	1.28		0.65		0.29
Pumpkinseed	0.15		2.66		0.34	0.29	1.13	0.30		0.74		0.49
White sucker	0.20		2.36	0.09	0.39	0.14	1.04	0.38		0.38		0.69
Lake trout			1.25		0.20	0.10	1.66	0.49	0.07	0.50		0.23
Freshwater drum			2.61		0.73		1.16	1.26				0.49
Alewife			1.59		0.18	0.31	2.04	0.87	0.11	0.16		0.14
Common shiner	0.15		6.04	0.06	0.37	0.06	2.01	0.40		0.60		0.63
White crappie			2.76		0.36		1.35	0.12		0.08		0.31
Walleye			1.76		0.25	0.03	1.01	0.42				0.20
Channel catfish	0.15		2.61		0.12		0.78	0.07		0.40		0.66
Greater redhorse			2.42		0.20	0.36	0.94	0.14		0.36		0.47
Black crappie			4.63		0.31	0.06	2.59	0.44		0.36		0.25
Smallmouth bass			3.30	0.06	0.28		1.39	0.23		0.46		0.37
Golden shiner			2.68		0.18		2.35	0.13		0.53		0.31
Slimy sculpin	0.52	0.26	1.78	0.13	0.21	0.13	0.67	0.24	0.12	0.21	0.03	0.20
Brown bullhead			2.58		0.30		1.60	0.07		0.33		0.60
Redbreast sunfish			5.68		0.27		2.43	0.29		0.80		0.84
Blacknose dace	0.43	0.24	1.87	0.27	0.16	0.22	0.48	0.21	0.10	0.72	0.03	0.48
Rock bass	0.25		2.83		0.18		1.31	0.13		0.40		0.36

Longnose dace	0.56		1.17	0.39	0.14	0.35	0.41	0.26	0.08		0.03	0.47
Fantail darter	0.58	0.21	1.87	0.27	0.25	0.21	0.73	0.41	0.10	0.25	0.01	0.31
Bowfin			1.46		0.50		1.04	0.41		0.34		0.34
Chain pickerel			2.35		0.20		1.77	0.52		0.23		0.23
White perch	0.13	0.16	1.88	0.05	0.24	0.05	1.46	1.18	0.15	0.39	0.22	
Burbot			1.06		0.27		0.46	0.17		0.35		0.22
Yellow perch	0.66		2.16	0.13	0.34		0.88	0.24		0.34		0.26
Bluegill			6.47		0.26		2.20	0.14		0.60		0.50
Mean CV (%)	37	38	18	32	44	49	23	35	50	52	65	18

* The position of double bonds for these fatty acids was not determined due to their low concentrations

Table S3c, continued

Fatty acid %wt	20:4n-6	20:4n-3	20:5n-3	22:4n-6	22:5n-6	22:5n-3	22:6n-3	24:5n-3	24:6n-3	Total PUFA
Rainbow smelt	5.72	1.46	9.97	0.26	3.83	2.24	26.44			58.98
Pumpkinseed	9.91	0.44	8.00	0.82	2.06	3.91	15.41	0.01	0.48	47.14
White sucker	11.64	0.55	8.59	1.60	1.46	2.99	14.69	0.18	1.08	48.46
Lake trout	5.44	0.97	7.25	0.58	2.80	3.09	24.61	0.12	0.06	49.43
Freshwater drum	16.15	0.53	14.11	1.37	1.15	4.23	4.95			48.73
Alewife	5.43	1.20	9.79	0.17	1.43	1.97	15.15	0.07	0.16	40.77
Common shiner	12.59	0.67	11.16	1.23		0.94	10.16	0.15	0.46	47.66
White crappie	15.15	0.55	5.77	0.98	2.13	2.95	14.65			47.15
Walleye	9.47	0.53	6.44	0.51	2.07	3.29	21.46			47.44
Channel catfish	8.46	0.61	8.18	0.65	1.80	3.08	15.66			43.23
Greater redhorse	5.84	1.02	15.43	0.42	1.11	5.12	14.41	0.29	0.72	49.24
Black crappie	17.17	0.94	9.14	0.54	0.65	5.87	11.49			54.44
Smallmouth bass	14.14	0.70	4.95	1.30	3.27	3.96	19.38	0.03	0.73	54.56
Golden shiner	10.49	0.73	12.40	0.47	0.96	3.62	16.74			51.59
Slimy sculpin	8.01	0.16	17.36	0.38	0.30	4.39	17.78	0.08	0.31	53.26
Brown bullhead	14.02	0.56	7.77	0.90	2.04	2.38	16.52			49.67
Redbreast sunfish	10.60	0.55	6.20	1.27	1.85	5.02	18.69			54.49
Blacknose dace	4.50	0.51	11.62	0.23	0.51	4.59	24.04	0.23	0.64	52.09
Rock bass	7.88	0.43	9.69	0.42	0.92	6.66	24.94			56.41
Longnose dace	3.47	0.04	16.80	0.17	0.35	5.55	22.93	0.50	0.74	54.41

Fantail darter	4.58	0.30	20.61	0.27	0.44	5.00	18.23	0.33	0.91	55.85
Bowfin	12.35	0.80	10.69	1.18	1.64	4.15	13.76			48.66
Chain pickerel	11.62	0.57	9.54	0.58	2.31	3.58	29.65			63.16
White perch	13.44	0.84	15.09	0.56	3.49	3.95	16.31			59.59
Burbot	21.37	0.32	7.37	2.31	4.86	2.42	13.90			55.09
Yellow perch	10.82	0.84	9.03	0.57	1.46	2.64	17.01	0.05	1.21	48.62
Bluegill	15.18	0.84	5.11	1.27	3.05	4.73	15.39			55.75
Mean CV (%)	13	17	13	29	28	22	14	35	43	6

Table S1.4a. Normal saturated FA of fish skin (weight %).

Fatty acid %wt	12:0	13:0	14:0	15:0	16:0	17:0	18:0	20:0	22:0	Total straight SFA
Rainbow smelt	0.10		3.33	0.70	20.21	0.65	6.00	0.54		31.53
Lake trout	0.17		2.09	0.65	23.04	0.64	7.02	0.18	0.05	33.84
Pumpkinseed	0.04	0.13	1.44	0.57	20.38	1.05	9.50	0.69	0.23	34.03
Alewife	0.23	0.05	4.90	0.82	23.73	0.97	4.83	0.20	0.18	35.91
White crappie			1.69	0.74	22.01	1.13	12.54	0.47		38.58
White sucker	0.12	0.02	1.61	0.54	19.78	0.87	6.67	0.26	0.14	30.02
Walleye	0.09	0.14	1.78	0.72	21.56	1.03	11.72	0.56		37.60
Brown bullhead			1.21	0.64	20.19	1.10	12.80	0.51		36.46
Rock bass	0.05	0.13	0.98	0.44	17.93	0.81		0.47		20.81
Greater redhorse	0.51		4.25	0.43	17.82	0.42	6.63	0.21		30.26
Black crappie	0.08	0.06	1.18	0.42	20.32	0.74	7.19	0.73		30.72
Freshwater drum			1.79	0.66	21.44	1.19	19.47	0.61		45.16
Redbreast sunfish			1.56	0.37	16.07	0.76	9.32	0.29		28.38
Slimy sculpin	0.69	0.05	2.50	0.46	14.88	0.34	6.12	0.55		25.60
Channel catfish			1.89	0.45	18.10	0.70	7.86	0.37		29.37
Smallmouth bass			1.77	0.59	18.88	0.78	8.57	0.50		31.08
Blacknose dace	0.27	0.03	2.99	0.24	14.11	0.33	4.70	0.52		23.20
Golden shiner			0.86	0.42	19.69	0.91	10.53	0.32		32.72
Chain pickerel			0.81	0.39	19.13	0.55	8.11	0.16		29.15
White perch	0.09	0.02	2.36	0.34	16.33	0.39	5.41	0.43		25.36

Fantail darter	0.10	0.02	2.29	0.34	17.93	0.34	6.74	0.21		27.98
Common shiner	0.18	0.03	1.62	0.41	20.92	0.56	6.13	0.19		30.03
Bowfin			1.00	0.45	17.77	0.57	7.84	0.09		27.72
Longnose dace	0.26	0.03	2.67	0.30	15.70	0.38	6.60	0.24	0.30	26.50
Yellow perch	0.06	0.07	2.17	0.62	21.75	0.79	8.53	0.45	1.12	35.56
Borbot			0.93	0.28	14.65	0.20	8.60	0.29		24.94
Bluegill			1.52	0.65	18.15	0.89	8.65	0.52		30.39
Mean CV (%)	58	34	21	18	8	20	20	46	73	9

Table S1.4b. Branched chain FA of fish skin (weight %).

Fatty acid %wt	*iso-* 13:0	*ai-* 13:0	*iso-* 14:0	*iso-* 15:0	*ai-* 15:0	*iso-* 16:0	*iso-* 17:0	*ai-* 17:0	*iso-* 18:0	*ai-* 19:0	*iso-* 20:0	Phytanic acid	Total BCFA
Rainbow smelt	0.09		0.10	0.70	0.33	0.35	0.44	0.85	0.23	0.10		2.02	3.20
Lake trout			0.02	0.37	0.13	0.24	1.09	0.75	0.41	0.14			3.16
Pumpkinseed	0.03		0.03	0.27	0.15	0.23	0.76	1.00		0.20	0.07	0.32	2.74
Alewife			0.09	0.79	0.48	0.20	0.31	0.34	0.15	0.07			2.44
White crappie				0.43	0.19	0.23	1.18	0.36					2.40
White sucker	0.04	0.01	0.04	0.34	0.17	0.25	0.50	0.63	0.08	0.16	0.08	0.44	2.28
Walleye		0.05	0.03	0.37	0.10	0.25	0.69	0.35		0.17	0.20		2.22
Brown bullhead			0.08	0.33	0.06	0.20	1.10	0.35			0.07		2.18
Rock bass				0.25	0.05	0.17	0.53	0.22	0.19	0.10	0.59	0.59	2.09
Greater redhorse			0.05	0.51	0.22	0.19	0.43	0.39		0.11		0.44	1.90
Black crappie			0.02	0.28	0.10	0.13	0.74	0.17	0.24	0.11			1.79
Freshwater drum				0.52	0.10	0.33	0.57	0.26					1.79
Redbreast sunfish				0.47	0.06	0.24	0.53	0.48					1.78
Slimy sculpin	0.06	0.03	0.08	0.53	0.24	0.21	0.34	0.24	0.02			1.00	1.76
Channel catfish				0.32	0.14	0.18	0.55	0.39		0.07	0.04	0.27	1.70
Smallmouth bass			0.07	0.36	0.11	0.20	0.49	0.34		0.06		0.54	1.63
Blacknose dace	0.04	0.03	0.07	0.37	0.18	0.20	0.28	0.37	0.03			0.38	1.56
Golden shiner			0.03	0.21	0.04	0.10	0.85	0.22					1.46
Chain pickerel				0.18	0.16	0.14	0.68	0.26					1.42

White perch	0.02	0.01	0.05	0.53	0.22	0.11	0.22	0.15	0.12			0.59	1.41
Fantail darter	0.01	0.02	0.03	0.26	0.12	0.24	0.36	0.28	0.04			0.46	1.34
Common shiner	0.04	0.02	0.04	0.27	0.20	0.17	0.30	0.23	0.06			0.14	1.34
Bowfin				0.25	0.06	0.12	0.51	0.32					1.25
Longnose dace	0.05	0.08	0.04	0.22	0.09	0.10	0.17	0.32	0.12			0.10	1.19
Yellow perch			0.03	0.30	0.08	0.17	0.28	0.22		0.08		0.37	1.16
Borbot				0.14	0.12	0.08	0.58	0.17					1.08
Bluegill				0.33	0.08	0.14	0.26	0.23					1.04
Mean CV (%)	54	59	45	24	34	26	27	24	56	29	70	37	20

Note *ai*: *anteiso*-methyl fatty acid; phytanic acid is excluded in total BCFA

Table S1.4c. Monounsaturated FA of fish skin (weight %).

Fatty acid %wt	14:1n-5	16:1n-7 [a]	17:1n-8	18:1n-9 [b]	20:1n-9	20:1n-7	22:1n-9	24:1n-9	Total MUFA
Rainbow smelt		4.20	0.35	9.03	0.75	0.26		0.69	15.28
Lake trout		6.63	0.62	20.33	0.55	0.12		1.05	29.30
Pumpkinseed	0.11	7.25	0.49	13.29	0.81	0.14		0.33	22.42
Alewife		7.74	0.41	20.27	0.97	0.22	0.16	0.62	30.39
White crappie		4.41		13.19	0.32	0.21		3.35	21.47
White sucker	0.08	14.27	0.77	18.22	0.63	0.14		0.32	34.44
Walleye		6.14		17.13	0.44	0.14		0.87	24.72
Brown bullhead		4.25		18.68	0.33	0.31		2.27	25.84
Rock bass		6.40	0.58	13.57	0.61	0.18		0.56	21.90
Greater redhorse	0.51	17.36	0.79	17.70	0.68	0.14		0.44	37.60
Black crappie	0.06	4.38	0.66	15.44	1.07	0.31		0.58	22.50
Freshwater drum		5.69		13.59	0.88			1.17	21.33
Redbreast sunfish		6.12		20.87	0.72			0.35	28.06
Slimy sculpin	0.32	14.29	1.32	16.68	1.01	0.22		0.11	33.95
Channel catfish		7.51	1.49	29.07	0.85	0.21		0.22	39.34
Smallmouth bass		5.38	0.70	12.65	0.85	0.31	0.03	1.01	20.94
Blacknose dace	0.31	13.35	0.47	19.54	0.76	0.56		0.22	35.21
Golden shiner		5.03		16.84	0.10			1.26	23.23
Chain pickerel		3.40		18.24	0.13	0.43		1.79	23.99
White perch	0.07	9.56	0.73	21.21	1.16	0.52		0.28	33.54

Fantail darter	0.14	12.53	1.06	10.75	0.16	0.12		0.14	24.89
Common shiner	0.15	8.33	0.65	19.09	0.38	0.06		0.01	28.67
Bowfin		7.66		25.29	0.35			1.12	34.42
Longnose dace	0.21	14.19		14.00	0.78	0.30	0.72	0.32	30.52
Yellow perch		7.31	0.97	11.58	0.38	0.10	0.15	1.95	22.43
Borbot		4.65		21.60	0.13			5.06	31.44
Bluegill		4.25		15.41	0.66	0.05		0.61	20.99
Mean CV (%)	54	18	37	12	51	53	44	45	11

a. Smaller amount of 16:1n-9 and 16:1n-5 coelute with 16:1n-7

b. Smaller amount of 18:1n-7 coelute with 18:1n-9

Table S1.4d. Polyunsaturated FA of fish skin (weight %).

Fatty acid %wt	16:2*	16:3*	18:2n-6	18:2*	18:3n-6	18:3*	18:3n-3	18:4n-3	20:2n-9	20:2n-6	20:3n-9	20:3n-6
Rainbow smelt			3.51		0.16		2.40	1.42		0.58		0.52
Lake trout			1.81		0.14	0.11	1.51	0.56	0.17	0.25		0.24
Pumpkinseed	0.27		2.39		0.38	0.15	0.99	0.25		0.69		0.44
Alewife			3.18		0.50	0.05	5.21	2.76	0.45	0.34		0.27
White crappie			2.58		0.41		0.88	0.18		0.26		0.36
White sucker	0.57		3.30	0.23	0.54	0.13	1.49	0.71		0.31		0.53
Walleye			2.11		0.22	0.26	1.10	0.28				0.27
Brown bullhead			2.55		0.23		1.89	0.12		0.41		0.64
Rock bass	0.51	8.13	3.09		0.25		1.00	0.25		0.52		0.40
Greater redhorse			3.57		0.42	0.62	2.02	0.82		0.26		0.44
Black crappie			3.52		0.48	0.04	1.87	0.62		0.97		0.27
Freshwater drum			2.67		0.69		0.63	2.38				0.29
Redbreast sunfish			4.66		0.28		2.06	0.58		1.17		0.92
Slimy sculpin	1.43	0.95	3.13	0.18	0.41	0.11	1.36	0.55	0.15	0.60	0.04	0.19
Channel catfish	0.29		3.59		0.12		1.53	0.24		0.43		0.53
Smallmouth bass	0.56		2.66	0.12	0.57		1.17	0.48		0.48		0.41
Blacknose dace	1.43	0.53	3.95	0.38	0.38	0.34	1.02	0.61	0.20	0.87	0.05	0.52
Golden shiner			3.74		0.22		3.15	0.28		0.41		0.46
Chain pickerel			1.80		0.16		0.94	0.29		0.20		0.23

White perch	0.32	0.53	2.46	0.11	0.43	0.03	2.32	2.02	0.13	0.75		0.28
Fantail darter	0.87	0.68	1.87	0.20	0.25	0.15	0.61	0.45	0.07	0.14	0.02	0.27
Common shiner	0.21		7.20	0.11	0.43	0.04	2.22	0.43		0.60		0.61
Bowfin			2.46		0.51		2.11	0.44		0.79		0.37
Longnose dace	1.41		1.76	0.48	0.22	0.40	0.64	0.61	0.07		0.04	0.44
Yellow perch	0.75		2.51	0.21	0.78		1.20	0.51		1.31		0.36
Borbot			0.75		0.50		0.28	0.07		0.34		0.22
Bluegill			5.54		0.31		1.90	0.32		0.59		0.67
Mean CV (%)	55	29	21	31	39	51	32	34	36	43	49	18

* The position of double bonds for these fatty acids cannot be determined due to their low concentrations

Table S1.4d, continued

Fatty acid %wt	20:4n-6	20:4n-3	20:5n-3	22:4n-6	22:5n-6	22:5n-3	22:6n-3	24:5n-3	24:6n-3	Total PUFA
Rainbow smelt	5.14	1.33	8.28	0.39	2.74	1.63	19.88			47.97
Lake trout	7.14	0.77	5.49	0.62	1.58	1.95	11.11	0.22	0.10	33.78
Pumpkinseed	10.30	0.19	6.68	1.39	1.73	3.66	11.76	0.06	0.40	41.72
Alewife	3.13	1.28	6.83	0.54	0.68	1.19	4.25	0.09	0.15	30.92
White crappie	11.82	0.44	4.02	1.68	2.80	3.11	9.27			37.80
White sucker	9.71	0.42	5.66	0.96	0.81	1.45	6.79	0.17	0.95	34.73
Walleye	9.66	0.33	5.07	0.99	1.52	1.83	11.94			35.58
Brown bullhead	10.57	0.47	4.55	1.56	1.45	2.79	8.34			35.56
Rock bass	9.13	0.35	8.82	0.96	0.75	6.68	15.18			56.02
Greater redhorse	3.94	0.74	8.10	0.58	0.46	2.43	4.50	0.29	0.60	29.79
Black crappie	11.65	0.63	5.93	0.96	0.60	6.79	10.70			45.04
Freshwater drum	9.18	0.15	4.88	2.09	0.83	3.84	4.09			31.72
Redbreast sunfish	11.17	0.37	4.80	2.09	1.19	3.53	8.96			41.78
Slimy sculpin	6.79	0.14	11.78	0.27	0.24	2.24	7.12		0.55	38.23
Channel catfish	6.90	0.61	4.72	0.89	0.99	2.28	6.21			29.34
Smallmouth bass	12.08	0.48	4.32	1.68	2.45	3.91	14.75	0.10	0.49	46.70
Blacknose dace	3.60	0.55	8.85	0.44	0.30	3.45	11.62	0.18	0.39	39.63
Golden shiner	10.25	0.61	9.27	0.75	1.09	3.07	9.30			42.60
Chain pickerel	14.17	0.20	4.91	1.73	2.71	2.54	15.56			45.44
White perch	7.65	0.64	9.48	0.90	1.39	2.51	7.15			39.10

Fantail darter	5.73	0.22	14.89	0.34	0.33	3.31	13.79	0.20	0.94	45.34
Common shiner	10.67	0.51	8.79	0.60		1.29	5.70	0.10	0.36	39.90
Bowfin	12.83	0.35	5.71	2.63	1.16	2.32	4.92			36.60
Longnose dace	3.94	0.06	12.84	0.30	0.42	4.44	12.93	0.39	0.67	42.05
Yellow perch	9.16	0.25	7.50	1.24	1.09	1.66	10.71	0.18	1.68	41.09
Borbot	13.11	0.16	3.25	2.80	4.67	1.57	14.83			42.55
Bluegill	13.24	0.52	6.04	2.63	1.54	3.37	11.07			47.74
Mean CV (%)	20	23	17	23	30	21	21	37	34	9

Table S1.5a. EPA (%) Analysis of Variance

	AIC	Degrees of Freedom. Model, Residuals	F-value	p-value	Post-Hoc Test Tukey HSD
Location	142.1	6, 20	4.9	< 0.01	Cascadilla > Adirondacks, Oneida, Whitney Point, Susquehanna
Habitat	142.8	1, 23	16.7	< 0.001	Stream > Lake
Guild	149.8	3, 23	3.8	< 0.05	Invertivores > Piscivores, Mixed Diets

Table S1.5b. Total BCFA (%) Analysis of Variance

	AIC	Degrees of Freedom. Model, Residuals	F-value	p-value
Location	34.3	6, 20	1.8	> 0.05
Habitat	36.1	1, 25	0.04	> 0.05
Guild	38.1	3, 23	0.60	> 0.05

Table S1.5c. DHA (%) Analysis of Variance

	AIC	Degrees of Freedom. Model, Residuals	F-value	p-value
Location	177.7	6, 26	0.625	> 0.05
Habitat	172.3	1, 25	0.004	> 0.05
Guild	170.8	3, 23	1.734	> 0.05

Table S2.1. Fatty acid composition (weight%±SD) of nātto

Fatty acids	Wt%
13:0	0.29±0.23
i14:0	0.23±0.05
14:0	0.26±0.20
i15:0	0.26±0.01
ai15:0	0.56±0.02
15:0	0.14±0.07
i16:0	0.33±0.04
16:0	16.86±1.50
16:1n-9	0.04±0.03
16:1n-7	0.11±0.02
i17:0	0.19±0.06
ai17:0	0.14±0.01
17:0	0.12±0.07
18:0	5.30±2.28
18:1n-9	10.21±2.07
18:2n-6	53.68±2.39
18:3n-3	9.82±0.58
20:0	0.41±0.27
20:1n-9	0.38±0.32
21:0	0.26±0.27
22:0	0.25±0.01
24:0	0.18±0.06

Table S2.2 Fatty acid composition (weight%±SD) of shrimp paste and dried Acetes

Fatty acids	Shrimp paste	Dried shrimp (Acetes)
12:0		0.07±0.01
i14:0		0.04±0.03
14:0	3.01±0.34	2.94±0.38
i15:0	0.27±0.04	0.13±0.01
ai15:0	0.09±0.04	0.22±0.01
15:0	1.08±0.04	0.85±0.11
i16:0	0.33±0.06	0.15±0.04
16:0	23.15±1.05	24.29±1.96
16:1n-7	9.43±0.55	10.56±0.91
i17:0	0.96±0.20	0.12±0.01
ai17:0	0.26±0.03	0.14±0.01
3,7,11,15-methyl-16:0	0.37±0.01	0.37±0.00
17:0	2.25±0.22	1.56±0.01
i18:0	0.37±0.10	0.39±0.11
18:0	7.33±0.36	6.42±0.06
18:1n-9[a]	9.34±0.01	12.22±0.02
18:2n-6	1.47±0.03	1.48±0.05
18:3n-6	0.20±0.17	0.35±0.03
i20:0	0.24±0.18	0.10±0.01
18:3n-3	1.12±0.12	0.60±0.01
20:0	0.87±0.23	0.83±0.11
18:4n-3	0.46±0.06	0.64±0.03

20:1n-11		0.13±0.04
20:1n-9	1.78±0.95[b]	1.49±0.05
20:1n-7		0.16±0.03
20:2n-6	0.85±0.71	0.88±0.13
20:3n-6		0.15±0.02
20:4n-6	3.06±0.12	4.11±0.29
22:0	0.70±0.24	
20:4n-3	0.73±0.21	0.26±0.02
22:1n-9		0.59±0.11
20:5n-3	16.63±0.10	16.72±0.86
i24:0	0.66±0.03	
22:4n-6		0.49±0.08
22:5n-6	0.63±0.02	0.13±0.03
24:0	0.17±0.13	
22:5n-3	0.79±0.21	0.53±0.16
22:6n-3	11.43±0.22	9.91±2.48

a Small amounts of 18:1n-7 coeluted with 18:1n-9

b 20:1n-11 and 20:1n-7 coeluted with 20:1n-9 and their areas were combined

Table S2.3. Fatty acid composition (weight%±SD) of miso, kimchi and douchi

Fatty acids	Miso	Kimchi	Douchi
12:0	<0.003	0.10±0.01	<0.003
i14:0	<0.003	0.06±0.01	<0.003
14:0	0.17±0.01	1.04±0.03	0.21±0.05
i15:0	0.20±0.00	0.04±0.01	0.01±0.01
ai15:0	<0.003	0.07±0.00	0.02±0.01
15:0	0.05±0.01	0.33±0.01	0.07±0.02
i16:0	0.04±0.00	0.12±0.01	0.01±0.00
16:0	8.57±0.14	21.62±0.07	12.19±0.30
e16:0	7.70±0.08	0.69±0.04	<0.003
16:1n-7	<0.003	1.11±0.04	0.21±0.01
i17:0	<0.003	0.08±0.00	0.02±0.00
ai17:0	0.10±0.03	0.21±0.02	0.01±0.00
17:0	0.10±0.00	1.96±0.11	0.04±0.02
17:1n-8	<0.003	0.09±0.01	0.07±0.00
i18:0	0.04±0.02	0.06±0.02	<0.003
16:3	<0.003	0.21±0.01	<0.003
18:0	3.76±0.01	3.80±0.01	7.38±0.18
18:1n-9	12.82±0.01	7.24±0.15	18.61±0.80
18:1n-7[a]	NA	4.20±0.20	NA
e18:1n-9	6.43±0.06	0.59±0.04	<0.003
e18:1n-7	<0.003	0.25±0.02	<0.003
18:2n-6	28.22±0.02	28.99±0.09	50.08±1.12
e18:2n-6	21.18±0.31	3.69±0.03	<0.003
18:3n-6	<0.003	<0.003	0.39±0.01

18:3n-3	4.94±0.01	17.31±0.02	10.11±1.73
20:0	0.09±0.01	0.07±0.01	0.28±0.02
e18:3n-3	3.36±0.03	1.43±0.04	<0.003
e20:0	0.10±0.01	<0.003	<0.003
20:1n-9	0.57±0.00	0.05±0.00	0.13±0.01
CLA	<0.003	0.34±0.11	<0.003
21:0	<0.003	0.57±0.04	<0.003
20:2	0.37±0.02	<0.003	<0.003
20:4n-6	<0.003	0.30±0.01	<0.003
20:3n-3	<0.003	0.10±0.04	<0.003
22:0	0.50±0.17	0.23±0.01	0.16±0.01
e22:0	0.72±0.00	<0.003	<0.003
22:1	<0.003	1.64±0.00	<0.003
20:5n-3	<0.003	0.26±0.02	<0.003
24:0	<0.003	0.46±0.05	<0.003
24:1	<0.003	0.05±0.01	<0.003
22:6n-3	<0.003	0.65±0.03	<0.003

a. For miso and douchi, 18:1n-7 coeluted with 18:1n-9

Table S3.1. Fatty acid composition of vernix, amniotic fluid, gastric contents, meconium and serum from late-term California sea lion fetuses.

	Vernix	Amniotic fluid	Gastric content	Meconium	Serum
iso-13:0	0.01	0.02	0.03	0.05	0.09
13:0	0.01	0.00	0.01	0.02	0.01
iso-14:0	0.00	0.00	0.00	0.01	0.00
14:0	1.96	1.62	1.13	0.91	1.43
iso-15:0	0.08	0.06	0.11	0.06	0.08
14:1	0.08	0.06	0.06	0.06	0.15
anteiso-15:0	0.06	0.00	0.00	0.00	0.00
15:0	1.04	0.63	0.50	0.68	0.64
iso-16:0	0.36	0.37	0.18	0.34	0.21
16:0	45.26	36.42	28.92	31.76	34.47
16:1n-9	1.82	1.64	1.17	0.54	1.13
16:1n-7	2.13	1.89	2.83	1.21	4.71
iso-17:0	0.28	0.27	0.16	0.25	0.35
anteiso-17:0	0.20	0.16	0.06	0.09	0.10
17:1	0.11	0.05	0.03	0.06	0.27
17:0	0.53	0.64	0.62	0.88	0.40
17:1n-8	0.26	0.23	0.44	0.66	0.16
17:1b	0.09	0.11	0.14	0.04	0.19
iso-18:0	0.97	1.00	0.79	1.12	0.18
18:0	8.83	8.90	13.06	17.64	7.96
18:1 isomers	13.25	18.09	21.67	13.06	25.54

19:0 branched	0.11	0.10	0.11	0.13	0.23
18:2	0.11	0.09	0.09	0.06	0.03
18:2n-6	0.44	0.38	0.63	0.40	0.43
19:0	0.89	0.66	0.65	0.92	0.37
18:3n-6	0.08	0.18	0.08	0.16	0.12
19:1 isomers	0.12	0.09	0.08	0.09	0.10
iso-20:0	3.50	4.60	2.30	4.74	0.01
20:1	0.06	0.16	0.04	0.08	0.00
20:0	1.44	1.70	0.92	2.77	0.10
20:1n-9	0.96	1.15	0.62	1.21	0.49
20:1n-7	0.36	0.40	0.23	0.27	0.23
iso-21:0	0.18	0.24	0.04	0.12	0.02
20:2n-9	0.40	0.37	0.16	0.23	0.26
anteiso-21:0	0.08	0.08	0.11	0.06	0.00
20:2n-6	0.68	0.81	0.32	0.37	0.51
21:0+20:3n-9	0.42	0.60	0.37	0.40	0.32
20:3n-6	0.10	0.14	0.19	0.18	0.12
iso-22:0	1.62	2.32	0.44	2.46	0.00
20:4n-6	3.59	5.13	11.20	4.58	10.32
22:1	0.08	0.09	0.05	0.05	0.00
22:0	0.75	0.79	0.47	1.68	0.03
22:1 isomers	1.09	1.63	1.02	0.60	1.18
iso-23:0	0.05	0.06	0.00	0.08	0.01
20:5n-3	0.12	0.10	0.22	0.04	0.31

anteiso-23:0	0.11	0.13	0.01	0.09	0.01
iso-24:0	0.38	0.63	0.42	0.70	0.21
22:4n-6	0.19	0.27	0.32	0.52	0.23
24:0	0.82	0.76	0.49	1.75	0.05
24:1	0.49	0.57	0.47	0.87	0.61
22:5n-3	1.58	1.69	1.92	2.11	3.13
22:6n-3	1.21	1.51	2.68	1.98	2.33
26:0	0.19	0.14	0.34	0.23	0.00
26:1 isomers	0.35	0.27	1.02	0.36	0.00
28:1a	0.00	0.00	0.00	0.07	0.00
28:1b	0.00	0.00	0.00	0.08	0.00

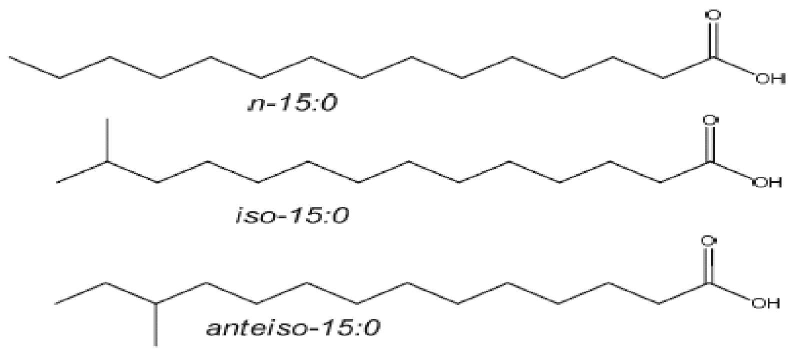

Figure S1.1. Comparison of straight chain, *iso*-pentadecanoic acid and *anteiso*- pentadecanoic. *Iso* BCFA terminate in a propan-2-yl (isopropyl) group and are not chiral around the tertiary carbon. *Anteiso* BCFA terminate in a butan-2-yl (*sec*-butyl) group and the tertiary carbon is chiral.

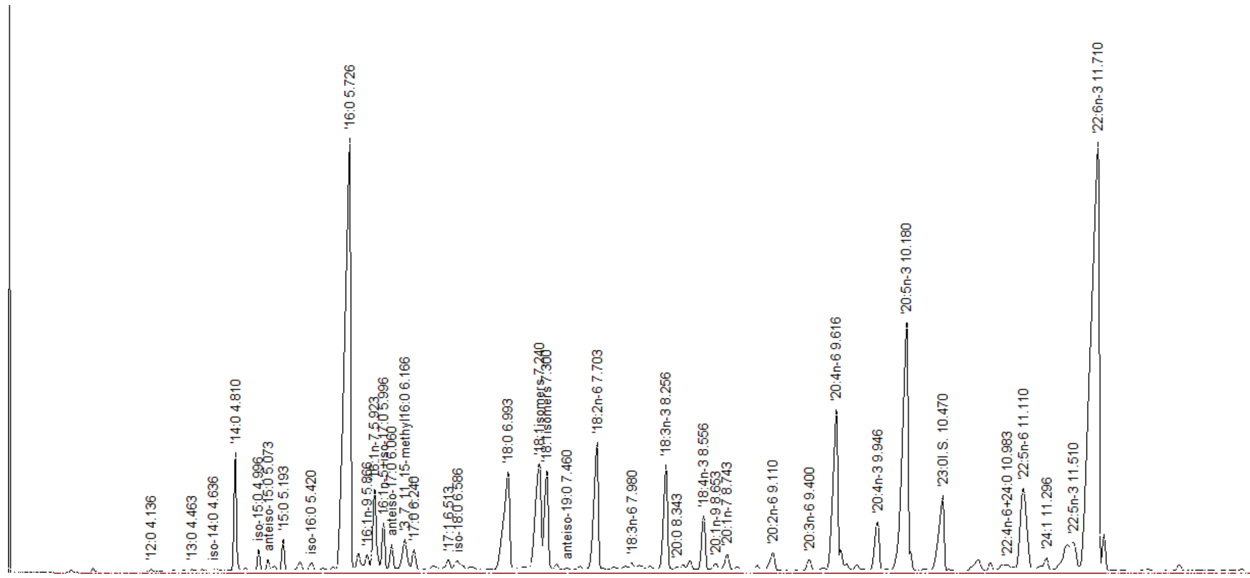

Figure S1.2a. A typical GC-FID chromatogram of fish muscle fatty acid profile.

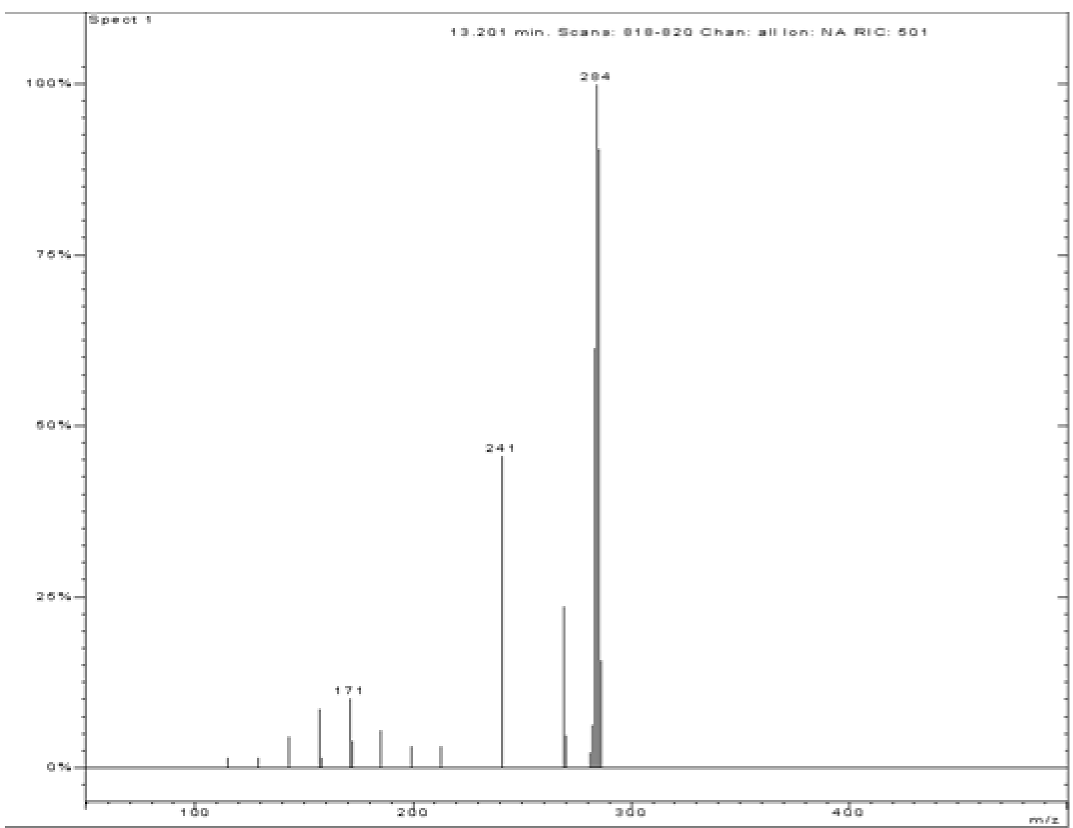

Figure S1.2b. A typical EIMS/MS chromatogram characteristic of *iso-17:0*.

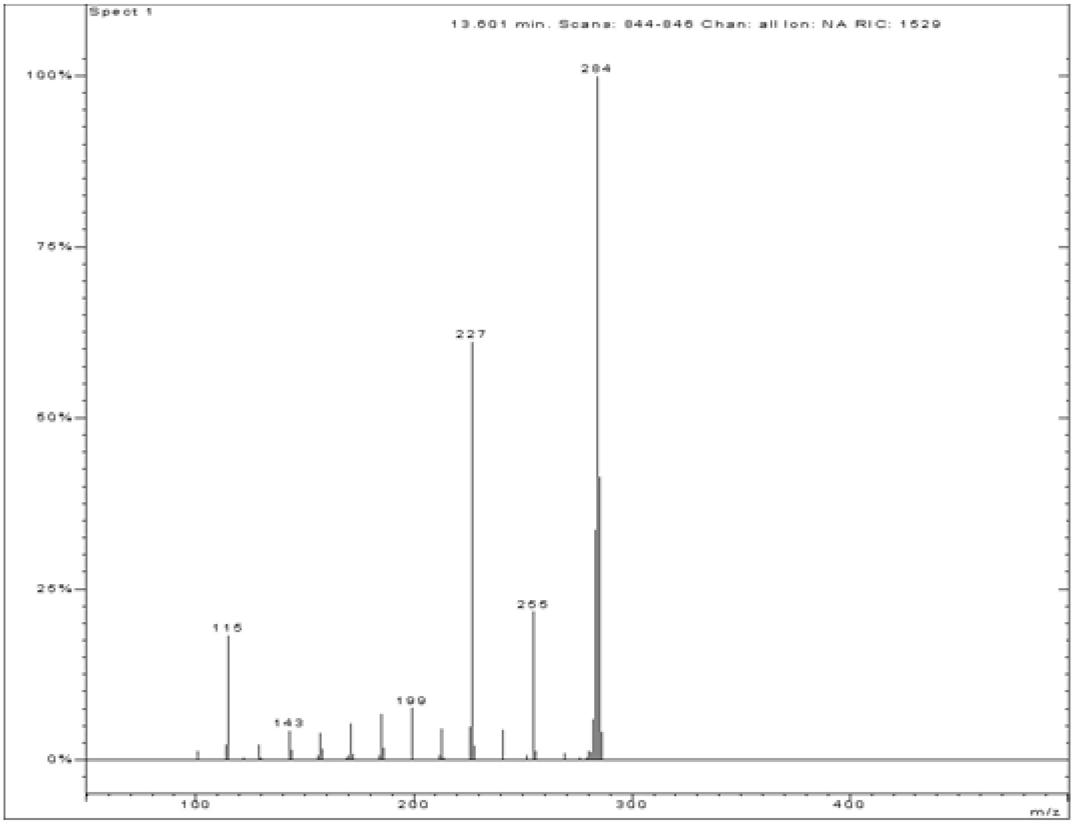

Figure S1.2c. A typical EIMS/MS chromatogram characteristic of *anteiso-17:0*.

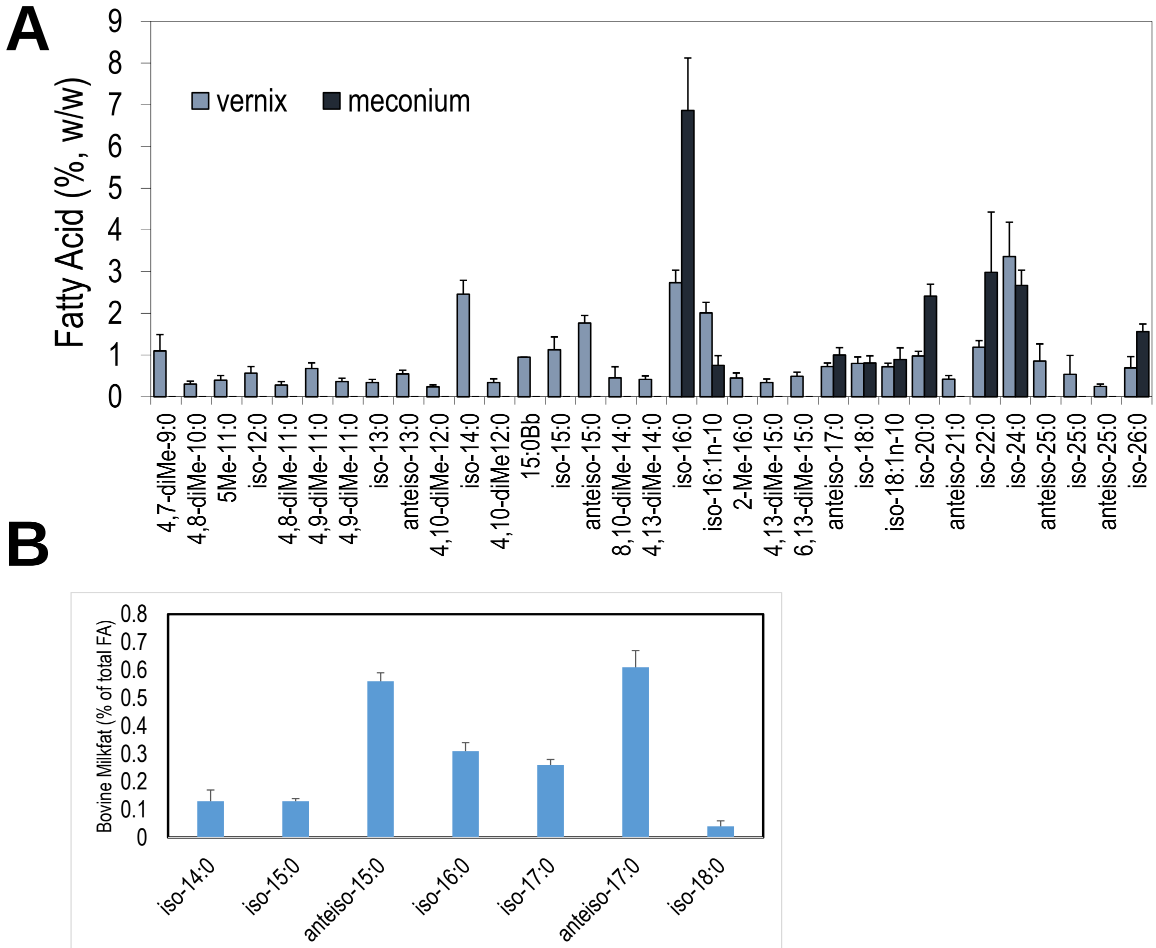

Figure S3.1. (A) Human vernix and meconium BCFA distributions[2]. (B) Cow milkfat BCFA distribution[3]

1. Smith, C. L., The inland fishes of New York State. The Department: Albany, NY :, 1985.
2. Ran-Ressler, R. R.; Devapatla, S.; Lawrence, P.; Brenna, J. T., Branched chain fatty acids are constituents of the normal healthy newborn gastrointestinal tract. *Pediatric research* **2008,** *64* (6), 605-9.
3. Ran-Ressler, R. R.; Sim, D.; O'Donnell-Megaro, A. M.; Bauman, D. E.; Barbano, D. M.; Brenna, J. T., Branched chain fatty acid content of United States retail cow's milk and implications for dietary intake. *Lipids* **2011,** *46* (7), 569-76.